.Walter Reed Army Institute of Research

Tropical Medicine Course

*** Laboratory Manual ***

Seventh Edition

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Preface to the Seventh Edition

The Walter Reed Army Institute of Research (WRAIR) was established by War Department General Orders No. 51, dated 24 Jan 1893 as the Army Medical School. Today, the WRAIR is part of the U.S. Army Medical Research and Materiel Command (USAMRMC), a subordinate command of the U.S. Army's Medical Command (MEDCOM). The WRAIR enterprise includes a laboratory in Silver Spring, MD and subordinate laboratories in Thailand, Kenya, and Germany; a new lab will open in the Republic of Georgia in FY15. The WRAIR is the Department of Defense's largest biomedical research facility and a National resource.

Tropical Medicine education initiatives began at the Army Medical School in July 1941 under BG Russell Callendar, Commandant. The course ran for 30 days and consisted of didactic and laboratory sessions, very similar to today's course. Over the next fifty years, the course changed in name and scope but maintained its dedication to educating military medical providers and medical support personnel in the tenets of tropical medicine and infectious diseases. In 1954, the Institute executed the, "Advanced Military Preventive Medicine Course," which was eventually supplanted in December 1966 by the, "Global Medicine Course." This 12 week course was divided into four weeks of "Epidemiology and Applied Biostatistics", three weeks of "Ecology and Disease", and five weeks of "Tropical Medicine". In February of 1972, the Global Medicine Course was split into a five week course called, "Military Medical Ecology," and a six week course called the, "Tropical Medicine Course." The first Tropical Medicine Course was offered in July and August of 1972 and was attended by 11 medical officers and four clinical clerks. The course endured until 1993.

The aggressive operational tempo during the wars in Afghanistan and Iraq, the increasing numbers and sustainment of operations in austere environments, and the formation of AFRICOM drove increasing educational needs and requirements of Special Operations Command

(SOCOM) medical providers and medical support personnel. In response, the WRAIR developed a one week course dedicated to teaching students how to recognize, diagnose, and manage infectious diseases common in the deployed OCONUS environment. A curriculum was developed focusing on COCOM-specific disease entities which adversely impact military operations and cause morbidity and mortality in U.S. Service Members. Students represent a broad spectrum of medical providers (physicians, physician assistants, nurses, nurse practitioners, and combat medics) and medical support personnel (entomologists, preventive medicine personnel, and environmental safety officers) from both military and non-military communities. Student diversity strengthens today's course.

This manual is intended to supplement the information provided during class and lab activities. It is not exhaustive but focused on high value information. Although course staff and faculty frequently update the content of the manual, the field of infectious diseases and tropical medicine is dynamic and new information becomes available with great frequency. The course faculty encourages you to explore the scientific literature for specific topics of interest.

The course is designed to provide the student with tools he or she requires to protect and sustain the health of those who serve our Nation through military and government service. The course directors seek continuous improvement and rely on our student's clear and direct feedback on what activities should continue and which require improvement or modification. Please support the course with your opinions and perspectives.

Thank you for your interest in infectious diseases and tropical medicine. I hope the course meets your expectations and positively influences how you practice medicine or otherwise support the same.

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List of Abbreviations and Acronyms

CAP	College of American Pathologists
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CHCS	Composite Health Care System
CHIKV	Chikungunya Virus
CL	Cutaneous Leishmaniasis
CLIA	Clinical Laboratory Improvement Amendments
CONUS	Continental United States
DENV	Dengue Virus
DHF	Dengue Hemorrhagic Fever
DSS	Dengue Shock Syndrome
DOD	Department of Defense
IATA	International Air Transport Association
ICAO	International Civil Aviation Organization
IND	Investigational New Drug
JE	Japanese encephalitis
LDL	Leishmania Diagnostic Laboratory
LF	Lymphatic Filariasis
MCL	Mucocutaneous Leishmaniasis
NCMI	National Center for Medical Intelligence
PBSS	Phosphate Buffered Saline Solution
OCONUS	Outside the Continental United States
OTSG	Office of The Surgeon General
POC	Point of Contact
SOP	Standard Operating Procedure
USAPHC	United States Army Public Health Command
USUHS	Uniformed Services University of the Health Sciences
VL	Visceral Leishmaniasis
WHO	World Health Organization
WRAIR	Walter Reed Army Institute of Research
WRNMMC	Walter Reed National Military Medical Center

Lecture Notes (Clinical Synopsis)

The next several pages of this manual are clinical synopsis for many tropical diseases. The intent is that the most important points are summarized in a succinct fashion to allow for a quick reference to the lectures given during the course. These summaries are intended to be 1-2 pages in length and serve as quick references only. For more information about the topic we recommend reviewing the lecture slides provided to you during the course. Of course, these short notes do not replace the lectures or the other more detailed references.

These lecture notes are organized in this similar manner:

Introduction

Clinical Syndrome

Diagnosis

Treatment

Epidemiology

Prevention

Malaria

Malaria is a mosquito-borne infectious disease of humans caused by eukaryotic protists of the genus *Plasmodium*.

** Four species of Plasmodium can infect and be transmitted by humans. Severe disease is largely caused by *Plasmodium falciparum*. Malaria caused by *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* is generally a milder disease that is rarely fatal. A fifth species, *Plasmodium knowlesi*, is a zoonosis that causes malaria in macaques but can also infect humans.

***Anopheles* is the principal vector for malaria (most common species of mosquito to transmit malaria).

Clinical Syndrome: Uncomplicated → fever, non-specific flu-like symptoms, GI (nausea, diarrhea, vomiting); Severe **(usually caused by *P. falciparum*)**→ prostration, mental status changes leading to unconsciousness (cerebral malaria), acute respiratory distress syndrome.

Diagnosis: Malaria blood film – thick and thin blood smears, rapid diagnostic tests (RDT's)

Treatment:

- *Artemether/lumefantrine* – 1 tablet; 3-day treatment schedule with a total of 6 oral doses initial dose at Dx followed by the 2nd dose 8 hours later, then 1 dose po bid for the following 2 days. Adult: 4 tablets per dose.
- *Atovaquone/proguanil* – 1 adult tablet; a 3 day treatment schedule: 4 tablets per dose daily for 3 consecutive days.
- Quinine sulfate/doxycycline – Quinine: 8 mg base/kg, doxy: 100mg tablets; Quinine: 2 caplets should be sufficient for adult dosing, doxy: 1 tablet q12 hours for 7 days.
- *Mefloquine* – One tablet; 3 tablets at Dx and 2 tablets in 6-12 hours

- ***Severe Malaria treatment options*** →*Quinidine gluconate plus doxy* – 6.25 base/kg loading dose IV over 1-2 hours, then 0.0125 mg base/kg/min continuous infusion for at least 24 hours. Doxy: same as above, if patient not able to take oral medication, give 100 mg IV every 12 hours and then switch to oral doxy as soon as patient can take oral meds. For IV use, avoid rapid administration. Treatment course – 7 days.
- *IV artesunate*- must be done under IND with instructions for preparation and administration by CDC only for hospitalized patients within the US.
- ***P. vivax and ovale treatment options*** → Chloroquine: 600 mg base po immediately, followed by 300 mg base po at 6, 24, and 48 hours.

  AND Primaquine… should test for G6PD deficiency before starting primaquine, (If G6PD negative or low levels (<6%) primaquine causes hemolysis. Prescribe **30 mg** base which equals 2 tablets qd x 14 days.

Epidemiology: Malaria causes approximately 250 million cases of fever and approximately one million deaths annually. It is prominent in tropical and sub-tropical regions such as Sub-Sahara Africa, Asia, and the Americas and more prevalent in rural regions as opposed to urban. This disease is prevalent in these regions due to the warm, consistent temperatures and moisture.

Prevention: Situational awareness (pre-travel preparation, assess malaria risk in geographical location, urban vs. rural), Avoid mosquito bites (personal protection, DEET, insecticide treated nets), compliance with chemoprophylaxis, seek early diagnosis and treatment.

Leishmaniasis

Leishmaniasis is a disease caused by protozoan parasites that belong to the genus *Leishmania* and is transmitted by the bite of certain species of sand fly (subfamily Phlebotominae).

** **Cutaneous** leishmaniasis is the most common form of leishmaniasis. **Visceral** leishmaniasis is a severe form in which the parasites have migrated to vital organs. Note that there are other forms not covered in this short review.

- Cutaneous: the most common form which causes a sore at the bite site, which heals anywhere from months to a year, leaving depressed, atrophic scar.
- Visceral: the most serious form of Leishmaniasis, and can be fatal.

Clinical Syndromes:

Cutaneous → Symptoms are primarily skin sores weeks to months after person has been bitten. Other symptoms can include → fever, damage to the spleen and liver, and anemia.

Visceral → fever, wasting (extreme weight loss), splenomegaly (large spleen – left side), hepatomegaly (large liver – right side), pancytopenia (bone marrow depressed)

Diagnosis: Clinical diagnosis, parasitologic diagnosis (amastigotes in a smear, promastigotes in culture, PCR assessment for *DNA), immunological diagnosis (serology rK30 dipstick assay)*

Cutaneous → biopsy/aspiration/scraping, touch prep, PCR, culture

Visceral → biopsy of bone marrow or spleen, touch prep, PCR, culture, immunologic: rK39 test

Treatment: *Cutaneous* Leish →

- Pentostam – 20 mg/kg IV x 10-20 days

Visceral Leish →

- Ambisome (liposomal amphotericin B) 3 mg/kg on days 1-5, 14 & 21 or
- Pentostam – 20 mg/kg IV x 28 days

Epidemiology: Leishmaniasis can be transmitted in many tropical and sub-tropical countries, mostly found in developing worlds. Leishmaniasis is found from rainforests in Central and South America to deserts in West Asia and the Middle East. It affects as many as 12 million people worldwide, with 1.5–2 million new cases each year.

Prevention: personal protective measures, vector control, reservoir control, immunoprophylaxis

Dengue

Dengue also known as "**breakbone fever"** is an infectious tropical disease caused by the dengue virus. It is transmitted by mosquitoes within the *Aedes* genus (principally *A. aegypti; A. albopictus* also a vector) and is not transmitted person-to-person. **Infection with one of four serotypes of dengue virus: -DEN1, DEN2, DEN3, DEN4

Clinical Syndrome: People infected with dengue are commonly asymptomatic or have only mild symptoms such as an uncomplicated fever. The incubation period ranges from 3–14 days, but most often it is 4–7 days.

Symptomatic dengue clinical syndromes → Dengue fever (DF), Dengue hemorrhagic fever (DHF), and Dengue shock syndrome (DSS)

DF (day 1)

- Abrupt onset high fever (~105 degrees) *5-7 days fever (biphasic)
- Rash (early flushlike rash may be placed by a macular/morbilliform rash. Late petechial).
- Chills, Arthralgias
- Severe headache, **retro-orbital pain**
- Lumbosacral pain

DF (day 2)

- Severe muscle, joint pain, Lassitude
- Nausea, vomiting
- Epistaxis
- PE: fever, generalized rash, relative bradycardia, generalized lymphadenopathy, petechial hemorrhages.

DHF

- Onset as per classical dengue
- Damage to blood and lymph vessels
- Defervescence followed by
    - Ascites, abdominal pain
    - Pleural effusion
    - Hemorrhagic manifestations
    - Central cyanosis

- Diaphoresis

DHF (2)

- Restlessness
- Abdominal pain
- Hemorrhage
- Tender hepatomegally, splenomegally
- Pleural effusions, perirenal effusions, hepatic, splenic, pericardial, peritoneal effusions
- Shock
  - Rapid, weak pulse
  - Pulse pressure (<20mmHg)
  - Unobtainable BP

DSS

- Fluid leak outside of blood vessels (lasts 1-2 days)
- Massive hemorrhage
- Shock
- Cyanosis, massive pleural effusions, ascites
- Narrowing pulse pressures
- Can be fatal

Diagnosis: tests used for the lab diagnosis of primary dengue infection → Viremia (culture), RT-PCR, IgM ELISA, IgG ELISA, PanBio duoCassette, Serum neutralization (PRNT)

Treatment: Supportive care, Intensive Care if necessary

Epidemiology*:* The incidence of dengue increased 30 fold between 1960 and 2010. The geographical distribution is around the equator with 70% of the total 2.5 billion people living in endemic areas from Asia and the Pacific. In the United States, the rate of dengue infection among those who return from an endemic area with a fever is 2.9–8.0% and it is the second most common infection after malaria to be diagnosed in this group

Prevention: Reduce exposure to vector by use of personal protective measures (DEET, permethrin treated uniforms, screened windows, mosquito netting) and local vector control (eliminate breeding sites, insecticides).

HIV

Human immunodeficiency virus (**HIV**) is a retrovirus that can cause acquired immunodeficiency syndrome (AIDS), a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections to thrive. Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate, or breast milk.

Clinical Syndrome: The stages of HIV infection are acute infection (also known as primary infection), latency and AIDS. Acute infection lasts for several weeks and may include symptoms such as fever, lymphadenopathy (swollen lymph nodes), pharyngitis (sore throat), rash, myalgia (muscle pain), malaise, and mouth and esophageal sores. The latency stage involves few or no symptoms and can last anywhere from two weeks to twenty years or more, depending on the individual. AIDS, the final stage of HIV infection, is defined by low CD4+ T cell counts (fewer than 200 per microliter), various opportunistic infections, cancers and other conditions.

Diagnosis: confirmatory testing is performed with a screening test, usually enzyme-linked immunosorbent assay (ELISA), followed by Western Blot.

Treatment: Treatment consists of highly active antiretroviral therapy, or HAART. Current HAART options are combinations (or "cocktails") consisting of at least three drugs belonging to at least two types, or "classes," of antiretroviral agents.

Epidemiology: Sub-Saharan Africa remains by far the worst-affected region, with an estimated 22.5 million people currently living with HIV (67% of the global total), 1.3 million deaths (72% of the global total) and 1.8 million new infections (69% of the global total). However, the number of new infections declined by 19% across the region between 2001 and 2009, and by more than 25% in 22 sub-Saharan African countries during this period. Asia is the second-worst affected region, with 4.9 million people living with HIV (15% of the global total).

Rickettsial Diseases

Rickettsial Diseases are caused by members of the genus *Rickettsia* (exception: agent of scrub typhus has recently been given status of its own genus, *Orientia*). *Coxiella* (agent of Q fever) and agents of Ehrlichiosis are close relatives of *Rickettsia*. Most rickettsial diseases are vectored by arthropods (mostly ticks).

Rickettsia = small, obligate intracellular bacteria
very difficult to grow in culture
By convention, divided into 2 groups:
Spotted Fever Group – *R. rickettsii, R. conorii, R. australis, R sibirica, R. akari, R. africae* and others
Typhus Group – *R. prowazekii, R. typhi, Orientia tsutusgamushi*

Epidemiology – All are arthropod-borne diseases often having a zoonotic (mammalian) reservoir. *R. prowazekii* (epidemic typhus) is an exception in that humans are the only reservoir. All rickettsioses are vectored by members of the order Acari (ticks and mites), except *R. prowazekii* (body louse) and *R. typhii* (flea). All except *R. prowazekii* have peak transmission in spring-summer when vectors and reservoirs are more abundant and active. Many are transmitted transovarially in vector, thus arthropods can be main reservoir as well.

Pathophysiology – After inoculation by an infected arthropod, *Rickettsia* disseminate to endothelial cells where they replicate and cause a systemic vasculitis. This underlying pathology is consistent throughout all rickettsioses, although severity and specific organ dysfunction varies.

Clinical Syndrome: The classic triad of ***fever, headache and rash*** should always trigger suspicion for rickettsial infection, especially in the proper epidemiologic setting. Many rickettsioses have an eschar (tache noir) at the site of initial inoculation. All begin with an influenza-like syndrome with high fever, headache, myalgia, malaise. Rash tends to be a later manifestation. Elevated liver associated enzymes (ALT, AST, GGT) are common as is leukopenia and thrombocytopenia.

Diagnosis: There is no reliable method for early diagnosis. Immunofluorescent antigen assay or PCR of skin biopsy of rash is available in reference labs. Otherwise diagnosis is made with acute and convalescent serology and takes weeks. Rapid improvement after doxy is relatively diagnostic.

Treatment: *Doxycycline! Doxycycline! Doxycycline!* If this is not an option, then appropriate second line therapy varies depending on disease, so you should look it up. Alternative agents include macrolides (esp. azithromycin), chloramphenicol, quinolones, rifamycins. ***Early treatment is the key to limiting morbidity and mortality – do not wait for confirmation!!!***

Prevention: Must focus on both vector and reservoir control as well as personal protective measures against arthopods. Personnel and clinicians should be aware of risks so that disease can be recognized and quickly treated.

Specific Rickettsial Diseases of Importance

Rocky Mountain Spotted Fever (RMSF)

(a.k.a. North American tick typhus, New World spotted fever, Sao Paolo fever)

Agent – *R. rickettsii*
Vector – dog tick (*Dermacentor*), *Amblyomma* (S.A.)
Reservoir – primarily ticks but also small mammals
Distribution – North and South America
Features – rash moves extremities => trunk, no eschar

Epidemic (Louse-Borne) Typhus

(a.k.a. classic typhus fever)
Agent – *R. prowazekii*
Vector – body louse (*Pediculus humanus*)
Reservoir - humans
Distribution – mostly Old World, common in refugee situations with breakdown in hygiene, more common in cold climates
Features – rash moves trunk => extremities, no eschar, altered mental status common, may recur later as Brill-Zinsser, reservoir in New World may be flying squirrels

Endemic (Murine) Typhus

(a.k.a. fleaborne typhus, shop typhus)
Agent – *R. typhi*
Vector – Oriental rat flea (*Xenopsylla cheopsis*),other fleas
Reservoir – rats, mice, other rodents
Distribution – worldwide
Features – less severe than classic typhus, no eschar

Mediterranean Spotted Fever

(a.k.a. Boutonneuse fever, Marseilles fever)
Agent – *R. conorii*
Vector – brown dog tick and various ticks
Reservoir – ticks, dog, rabbit, rodents
Distribution – Mediterranean basin, North and Central Africa, Middle East
Features- usually **single tache noir**

South African Tick Typhus

Agent – *R. africae* (some overlap with *conorii* infections)
Vector – *Amblyomma* ticks
Reservoir – ticks, large mammals (cattle, rhino)
Distribution – sub-Saharan Africa
Features – **frequently multiple tache noir**

Scrub Typhus

(a.k.a. miteborne typhus, Tsutsugamushi disease)
Agent – *O. tsutsugamushi*
Vector – trombiculid mite
Reservoir – mites
Distribution – East and S.E. Asia, in pockets west to Pakistan
Features – eschar generally in groin or axilla, frequently associated with adenopathy, can cause severe pneumonia, short-lived immunity

Multiple other rickettsial diseases or regional importance – you name a place and it likely has its own spotted fever!

Q Fever

A zoonotic infection with the rickettsial organism *Coxiella burnetti* causing an acute (or sometimes chronic) febrile illness. The disease was first described by E. H. Derick, who in 1935 investigated an outbreak of a febrile illness in meat packers in Queensland, Australia. He used the term Q (for query) fever to describe the illness, thus coining its present name.

Epidemiology: Common commensal organism of arthropods, fish, birds, various mammals (including rodents and marsupials); most important reservoirs are domestic ungulates (sheep, cows, goats); Ticks or other arthropods may be important in maintaining enzootic cycles; *C. burnetti* shed in feces, urine, milk and especially in birth products (10^9 organsims/gram); *Extremely* infectious – inoculation of **one** organism can cause human infection; Very hardy – has spore form that can survive in environment for months to years; Inoculation generally occurs in lungs by aerosol route; At risk = veterinarians, farmers (of livestock), abattoir workers and other animal handlers; can also result from indirect exposure, due to organism's high infectivity. *Examples:* persons handling contaminated laundry or persons in room with infected parturient cat.

Clinical Syndrome: Self-limited febrile illness → Most common syndrome with mild to relatively severe illness of fever, headache and constitutional symptoms lasting 5-10 days (incubation period 14-39 days), Can occasionally cause prolonged fever (> 2 weeks), pneumonia → next most frequent syndrome, ranges from incidental infiltrate on x-ray to severe multi-lobar pneumonia, often accompanied by GI symptoms.

Diagnosis: *Acute diagnosis is clinical* – serology usually used to confirm (or diagnose) later, serologic dx generally requires paired acute/convalescent sera, single complement fixation antibody (CF) titer ≥ 1:16 or IFA antibody titer ≥ 1:256 can be used for dx in proper clinical setting, *Common lab findings* → variable WBC (low or high), mild thrombocytopenia, mild liver-associated enzyme elevation. Chest x-ray often abnormal even if no clinical symptoms of pneumonia

Treatment: Acute infection generally resolves without treatment, Treatment of ongoing infection or pneumonia generally tetracyclines, fluroquinolones, macrolides – can combine any of these with rifampin, Treatment of endocarditis or chronic infection may require months-years of a combination of antibiotics

Bartonellosis

Bartonella is a genus of small, fastidious, Gram-negative bacteria related to *Brucella* and more distantly *Rickettsia*. Of the eight confirmed members of the genus, three are established causes of human disease: *B. henselae*, *B. quintana* and *B. bacilliformis*. These three organisms cause several diseases of relative epidemiologic uniqueness.

Pathogenesis

Much of the mechanisms of pathogenesis for *Bartonella* species remain a mystery. Our present knowledge indicates that most significant effects occur within the vasculature. *B. bacilliformis* is the only known pathogenic bacterium to invade human erythrocytes. *B. quintana, B. henselae* and *B. bacilliformis* have been shown to invade endothelial cells and replicate. All three species elaborate factors that interact with endothelial cells, stimulating proliferation of small vessels.

Diagnosis: Direct microscopic examination of Giemsa-stained blood smears will reveal abundant intra-erythrocytic Gram-negative bacilli in Oroya fever (*B. bacilliformis*). Diagnosis of other diseases caused by *Bartonella* is more difficult. *Bartonella* are difficult to grow in culture, although modern broth culture techniques (i.e. Bactec) are able to grow the organisms in 7 days (works better at temp of 28^{o}C). PCR is sometimes useful – particularly for *B. henselae*. Serologic tests are relatively reliable but do have problems with cross-reactivity with *Chlamydia* spp. and *Coxiella*, as well as between *Bartonella* species.

Cat Scratch Disease

Agent: *B. henselae* – Commensal bacteria in cats, the majority of young cats have intermittent *B. henselae* bacteremia without symptoms.

Epidemiology: Worldwide. Occurs in persons (young >> old) with cat exposure. Cat flea is probable vector.

Clinical Manifestations: Single or multiple popular/pustular lesions at inoculation sites. Pronounced regional lymphadenopathy (primarily upper extremities or neck)

accompanies. Low-grade fever and constitutional symptoms are not uncommon. Symptoms resolve in 1 – 7 weeks.
Complications: Rare cases of neurologic disease, retinitis, endocarditis. Almost all patients recover without problems, but immunocompromised have more complications.
Treatment: Controversial – most cases require no therapy (azithromycin has been shown to speed time to resolution, however). Severe cases or those with complications may benefit from doxycycline or a macrolide (with or without rifampin) or a quinolone.

Bacillary Angiomatosis
Agent: *B. henselae*, rarely *B. quintana*
Epidemiology: Affects immunocompromised (almost all late-stage HIV) exclusively, probably transmitted by cats (through fleas?).
Clinical manifestations: A progressive disease of proliferating neo-vascular nodules in skin, soft tissue and internal organs. Fever and constitutional symptoms often accompany disease when internal organs are involved.
Complications: This is a progressive, fatal disease in the immunocompromised unless treated.
Treatment: Long term doxycycline or macrolide (potentially for life). Key in HIV is to give HAART to restore immune function.

Isolated Culture-Negative Endocarditis
Agent: any *Bartonella*, usually *B. quintana* or *B. henselae*
Causes up to 3% of cases of "culture-negative" endocarditis (varies by study) – probably distributed worldwide. Treated with same medications as above.

Trench Fever (shinbone fever, quintan fever, Volhynia fever, Meuse fever)
Agent: *B. quintana*
Epidemiology: Disease of poor sanitary conditions worldwide, particularly affected troops in trenches in WWI. Vector is human body louse (*Pediculus humanus*). Recently has been noted in inner-city homeless.

Clinical manifestations: Self-limited disease of fever chills and constitutional symptoms. Sometimes accompanied by rash, conjunctivitis, headache, hepatosplenomegaly, vertigo, nystagmus. Usually lasts 4-5 days, often recurs in periodic episodes (anywhere from 3 – 8) of fever that last 5 days (thus quintan fever). Rarely occurs as long febrile illness of 2 – 6 weeks duration.
Complications: Endocarditis - generally occurs only in homeless and others who are relatively immunocompromised.
Treatment: doxy or macrolide (+/- rifampin)

Tropical Bartonellosis (Oroya fever, Andean bartonellosis, Carrion's disease)
Agent: *B. bacilliformis*
Epidemiology: Confined to foci in Andes. Vector is *Phlebotomus* sandfly and reservoir probably humans. Disease in native populations mostly in children, but spectacular outbreaks with high mortality can occur in visiting non-native populations (such as deployed troops).

Clinical manifestations: A biphasic illness (although each can occur without the other) consisting of an initial, acute, severe febrile illness with anemia associated with high mortality (40-90% fatality untreated) followed by a chronic cutaneous illness of vascular, wartlike nodules called *verruga peruana.* **Remember – bacilli are visible in RBC's!**

Complications: Most deaths are due to endocarditis, severe anemia or superimposed *Salmonella* (typhoid) infection.

Treatment: chloramphenicol (also gets *Salmonella*), doxy, macrolides, penicillins, streptomycin

TUBERCULOSIS:

TB is a common and potentially lethal infectious disease caused by various strains of mycobacteria, usually Mycobacterium tuberculosis in humans. It is spread through the air when people who have an active TB infection cough, sneeze, or otherwise transmit their saliva through the air. The main cause of TB, Mycobacterium tuberculosis (MTB), is a small aerobic non-motile bacillus.

Active Pulmonary TB →

Clinical syndrome: The classic symptoms are a chronic cough with blood-tinged sputum, fever, night sweats, and weight loss (the last giving rise to the formerly prevalent colloquial term "consumption").

Diagnosis: TST or IGRA usually positive, chest radiograph may be abnormal, respiratory specimens may be smear or culture positive (3 sputum smears if symptomatic).

Treatment: "4 for 2 and 2 for 4" INH, RIF, PYR, EMB x 2 months, INH, RIF x 4 months.

Prevention: Avoid droplets from active TB case

Infection control: Administrative controls – Airborne, droplet, nuclei precautions; Engineering controls (ventilation) – isolation, negative pressure rooms; Personal respiratory protection

Latent Tuberculosis →

Clinical Syndrome*:* no symptoms or physical findings suggestive of TB disease

Diagnosis: TST or IGRA positive, negative chest radiograph

Treatment: Isoniazid for daily for 9 months; (alternate regimens: Isoniazid 2x weekly for 9 months, Isoniazid daily for 6 months, Isoniazid 2x weekly for 6 months, Rifampin daily for 4 months).

Prevention: Avoid droplets from active TB case

VIRAL HEMMORHAGIC FEVERS

***Clinical Syndrome*:** acute, febrile, multisystemic illness characterized by malaise, myalgia, prostration, and bleeding diathesis

***Diagnosis*:** clinical pathology →

- thrombocytopenia or abnormal platelet function
- leucopenia
- some patients have anemia
- most have elevated liver enzymes
- Bilirubin is elevated in RVF and YF
- Prothrombin time, activated partial thromboplastin time (APTT) and bleeding time are prolonged
- Some have disseminated intravascular coagulation (DIC), those that have DIC have elevated d-dimers (FDP's) and decreased fibrinogen

Lab confirmation →

- Gold standard – virus isolation from blood, serum or tissue biopsy (BSL-4 lab)
- Electron microscopy
- Reverse transcription – polymerase chain reaction (increasingly important tool).
- Rapid ELISA techniques most easily employed
    - Antigen capture detection
    - IgM or IgG antibody capture
- Serology on paired sera
- Immunohistochemistry (IHC) and in situ hybridization (ISH) of infected tissue
    - Formalin-fixed tissue
    - CDC has developed a skin biopsy procedure for detection of EBOV using IHC

***Prevention/infection control*:**

- Standard precautions in initial assessments
- Private room upon initial hospitalization
- Barrier precautions
- Negative pressure rooms
- Airborne precautions if prominent cough, vomiting, diarrhea, hemorrhage

BABESIA

Babesia is a protozoan parasite of the blood that causes a hemolytic disease known as Babesiosis. The Babesia parasites in red blood cells closely resemble and are often confused with Malaria parasites. There are several species which can infect humans and all are transmitted by ticks, particularly those of the genus Ixodes.

Clinical Syndrome: For 25% of cases in adults and half of cases in children, the disease is asymptomatic or mild with flu-like symptoms. In cases of symptomatic infection, symptoms are characterized by irregular fevers, chills, headaches, general lethargy, pain and malaise. In severe cases, hemolytic anemia, jaundice, shortness of breath, and hemoglobinuria are documented due to the lytic effects of parasitic multiplication

Diagnosis: Wright-stained or Giemsa-stained peripheral blood smear reveals intraerythrocytic parasites (ring forms with a central pallor) and, rarely, pathognomonic tetrads of budding trophozoites, the so-called Maltese cross. To supplement a blood smear, diagnoses should be made with an indirect fluorescent antibody (IFA) test or PCR.

Treatment: The standard treatment has been clindamycin and quinine, but this regimen occasionally fails and patients report frequent side effects including tinnitus, decreased hearing, and diarrhea. Because of this, the drug regimen consisting of atovaquone and azithromycin is now the first line of treatment for mild/moderate disease.

Epidemiology: Of the species to infect humans, *B. microti* is most common in the Americas whereas *B. divergens* is the predominant strain found in Europe. Endemic areas are regions of tick habitat, including the forest regions of the Northeastern United States and temperate regions of Europe.

Prevention: The most effective public health measure for *Babesia* is avoidance of tick exposure.

TRYPANOSOMES

Trypanosoma is a genus of kinetoplastids, a group of unicellular parasitic flagellate protozoa. The majority of species are transmitted by blood-feeding invertebrates, but there are different mechanisms among the varying species. Then in the invertebrate host they are generally found in the intestine and normally occupy the bloodstream or an intracellular environment in the mammalian host. African trypanosomiasis is commonly known as Sleeping Sickness in humans and Nagana (meaning 'loss of spirit' in the Zulu language) in cattle.

African Trypanosomiasis

African Trypanosomiasis is an illness endemic to sub-Saharan Africa. It is caused by the flagellate protozoan *Trypanosoma brucei*, which exists in 2 morphologically identical subspecies: *Trypanosoma brucei rhodesiense* (East African or Rhodesian African trypanosomiasis) and *Trypanosoma brucei gambiense* (West African or Gambian African trypanosomiasis). Both of these parasites are transmitted to human hosts by bites of infected tsetse flies (*Glossina palpalis* transmits *T. brucei gambiense* and *Glossina morsitans* transmits *T. brucei rhodesiense*), which are found only in Africa.

Clinical Syndrome: The symptoms of East African trypanosomiasis develop more quickly (starting 1 mo after bite) than the symptoms of West African trypanosomiasis, which can begin months to a year after the first bite.
Both types of African trypanosomiasis cause the same generalized symptoms, including intermittent fevers, rash, and lymphadenopathy. Notably, individuals with the East African form are more likely to experience cardiac complications and develop CNS disease more quickly, within weeks to a month. The CNS manifestations of behavioral changes, daytime somnolence, nighttime insomnia, stupor, and coma result in death if untreated.

Diagnosis: A definitive diagnosis of infection requires actual detection of trypanosomes in blood, lymph nodes, CSF, skin chancre aspirates, or bone marrow.

Treatment: The type of drug treatment used depends on the type and stage of African trypanosomiasis (sleeping sickness). As treatment medicines are difficult to obtain and can be quite toxic, treatment should only be initiated after consultation with an experienced Infectious Diseases physician. IV Suramin or IM Pentamidine may be indicated for the hemolymphatic stage, while IV Melarsoprol or IV Eflornithine may be indicated for the neurologic stage.

Epidemiology: The major epidemiology factor in African trypanosomiasis is contact between humans and tsetse flies.

American Trypanosomiasis (aka Chagas Disease)
Chagas disease, also known as American trypanosomiasis, is caused by infection with the protozoan parasite *Trypanosoma cruzi*. New cases of vector-borne *T. cruzi* infection usually occur in persons who live in primitive houses in endemic areas. The living quarters are invaded by infected triatomines (kissing bugs), which become domiciliary. Infected triatomine insects take blood meals from humans and their domestic animals and deposit parasite-laden feces. The parasites are then transmitted via contact with breaks in the skin, mucosal surfaces, or the conjunctivas. Transmission can also occur congenitally or via blood transfusion or organ transplantation.

Clinical Syndrome: 3 phases of natural disease

Acute Phase: In most instances, a specific diagnosis is not made because of the nonspecific nature of the signs and symptoms. Acute Chagas disease carries a mortality rate of less than 5%. Death in the acute phase is typically caused by myocarditis and, less commonly, by meningoencephalitis.

Indeterminate Phase: By definition, the indeterminate phase of Chagas disease does not cause any symptoms.

Chronic symptomatic Chagas Disease: Ten to 30% of persons with chronic Chagas disease develop clinical manifestations of the disease. The most common and serious problems are cardiac, which are caused by an inflammatory cardiopathy that results from the persistence presence of the parasites in the heart. The gastrointestinal symptoms associated with chronic T cruzi infection typically result from denervation of hollow viscera and consequent dysfunction.

Diagnosis: The diagnosis of acute Chagas disease, which includes congenital Chagas disease and reactivation of chronic T cruzi infection in immunosuppressed persons, is based on direct detection of the parasites. In contrast, the diagnosis of chronic infection (indeterminate or chronic symptomatic phases) is generally based on serologic testing, since the low level of circulating parasites precludes microscopic detection.

Treatment: depends on phase of disease

Acute Phase: All patients with acute Chagas disease, including those with congenital infection and those with reactivation of chronic infections due to immunosuppression, should be treated with either benznidazole or nifurtimox.

Indeterminate Phase: All children with chronic T cruzi infection should receive either benznidazole or nifurtimox. In contrast, the probability of parasitologic cure with full courses of either drug in adults with long-standing T cruzi infection, most of whom were infected while quite young, is less than 10%

Chronic Chagas: The consensus among experts is that persons who have already developed cardiac or gastrointestinal symptoms should not be given antiparasitic treatment.

Prevention: Avoidance of infected triatomines (kissing bugs). The best way to prevent exposure is not to sleep or dwell in primitive (earthen-walled) houses in endemic areas.

ENTAMOEBA HISTOLYTICA/DISPAR

Entamoeba histolytica is an anaerobic parasitic protozoan, part of the genus Entamoeba. Predominantly infecting humans and other primates, *E. histolytica* is estimated to infect about 50 million people worldwide. Mammals such as dogs and cats can become infected transiently, but are not thought to contribute significantly to transmission.

Clinical Syndrome: Symptoms can include fulminating dysentery, bloody diarrhea, weight loss, fatigue, abdominal pain, and amoeboma. The amoeba can actually 'bore' into the intestinal wall, causing lesions and intestinal symptoms, and it may reach the blood stream. From there, it can reach different vital organs of the human body, usually the liver, but sometimes the lungs, brain, spleen, etc. A common outcome of this invasion of tissues is a liver abscess, which can be fatal if untreated.

Diagnosis: It can be diagnosed by stool samples, but it is important to note that certain other species are impossible to distinguish by microscopy alone. Trophozoites may be seen in a fresh fecal smear and cysts in an ordinary stool sample. ELISA or RIA can also be used.

Treatment: Metronidazole for the invasive trophozoites plus a lumenal amoebicide for those still in the intestine. Paromomycin (Humatin) is the luminal drug of choice, since Diloxanide furoate (Furamide) is not commercially available in the USA or Canada (being available only from the Centers for Disease Control and Prevention). A direct comparison of efficacy showed that Paromomycin had a higher cure rate.[10] Paromomycin (Humatin) should be used with caution in patients with colitis, as it is both nephrotoxic and ototoxic. Absorption through the damaged wall of the intestinal tract can result in permanent hearing loss and kidney damage.

> *Recommended dosage: Metronidazole 750 mg tid orally, for 5 to 10 days followed by Paromomycin 30 mg/kg/day orally in 3 equal doses for 5 to 10 days or Diloxanide furoate 500 mg tid orally for 10 days, to eradicate lumenal amoebae and prevent relapse.

GIARDIA LAMBLIA

Giardia lamblia is a flagellated protozoan parasite that colonizes and reproduces in the small intestine, causing giardiasis. The giardia parasite attaches to the epithelium by a ventral adhesive disc, and reproduces via binary fission. Giardiasis does not spread via the bloodstream, nor does it spread to other parts of the gastro-intestinal tract, but remains confined to the lumen of the small intestine. Giardia trophozoites absorb their nutrients from the lumen of the small intestine, and are anaerobes. Giardia infects humans, but is also one of the most common parasites infecting cats, dogs, beavers, and birds.

Clinical Syndrome: Symptoms of infection include diarrhea, malaise, excessive gas (often flatulence or a foul or sulphuric-tasting belch, which has been known to be so nauseating in taste that it can cause the infected person to vomit), steatorrhoea (pale, foul smelling, greasy stools), epigastric pain, bloating, nausea, diminished interest in food, possible (but rare) vomiting which is often violent, and weight loss. Pus, mucus and blood are not commonly present in the stool. It usually causes "explosive diarrhea" and while unpleasant, is not fatal. In healthy individuals, the condition is usually self-limiting, although the infection can be prolonged in patients who are immunocompromised.

Diagnosis: Accurate diagnosis requires an antigen test or, if that is unavailable, an ova and parasite examination of stool. Multiple stool examinations are recommended, since the cysts and trophozoites are not shed consistently.

Treatment: Human infection is conventionally treated with metronidazole, tinidazole or nitazoxanide.

Prevention: Boiling suspect water for one minute is the surest method to make water safe to drink and kill disease-causing microorganisms like Giardia lamblia if in doubt about whether water is infected with the Giardia parasite.

HELMINTHS

Helminths are a division of eukaryotic parasites that, unlike external parasites such as lice and fleas, live inside their host. They are worm-like organisms that live and feed off living hosts, receiving nourishment and protection while disrupting their hosts' nutrient absorption, causing weakness and disease. Those that live inside the digestive tract are called intestinal parasites. They can live inside humans as well as other animals.
Parasitic worms are categorized into three groups: cestodes (tapeworms), nematodes (roundworms), and trematodes (flukes).

CESTODES (the tapeworms)

Cestoda (Cestoidea) is the name given to a class of parasitic flatworms, commonly called tapeworms, of the phylum Platyhelminthes. Its members live in the digestive tract of vertebrates as adults, and often in the bodies of various animals as juveniles. Over a thousand species have been described, and all vertebrate species can be parasitised by at least one species of tapeworm. Several species parasitise humans after being consumed in underprepared meat such as pork (*T. solium*), beef (*T. saginata*), and fish (*Diphyllobothrium* spp.), or in food prepared in conditions of poor hygiene (*Hymenolepis* spp. or *Echinococcus* spp.).

FILARIAL WORMS

Filariasis is a parasitic disease and is considered an infectious tropical disease, that is caused by thread-like filarial nematodes (roundworms) in the superfamily Filarioidea, also known as "filariae".
Clinical Syndrome: The most spectacular symptom of lymphatic filariasis is elephantiasis—edema with thickening of the skin and underlying tissues—which was the first disease discovered to be transmitted by mosquito bites. Elephantiasis results when the parasites lodge in the lymphatic system. Elephantiasis affects mainly the lower extremities, while the ears, mucus membranes, and amputation stumps are affected less frequently. However, different species of filarial worms tend to affect different parts of the body: *Wuchereria bancrofti* can affect the legs, arms, vulva, breasts, and scrotum, while *Brugia timori* rarely affects the

genitals. Interestingly, those who develop the chronic stages of elephantiasis are usually amicrofilaraemic, and often have adverse immunological reactions to the microfilaria, as well as the adult worm.

The subcutaneous worms present with skin rashes, urticarial papules, and arthritis, as well as hyper- and hypopigmentation macules. *Onchocerca volvulus* manifests itself in the eyes, causing "river blindness" (onchocerciasis), the second leading cause of blindness in the world. Serous cavity filariasis presents with symptoms similar to subcutaneous filariasis, in addition to abdominal pain, because these worms are also deep tissue dwellers.

Diagnosis: Filariasis is usually diagnosed by identifying microfilariae on Giemsa stained thin and thick blood film smears, using the "gold standard" known as the finger prick test. The finger prick test draws blood from the capillaries of the finger tip; larger veins can be used for blood extraction, but strict windows of the time of day must be observed. Blood must be drawn at appropriate times, which reflect the feeding activities of the vector insects.

Treatment: The recommended treatment for patients outside the United States is albendazole (a broad spectrum anthelmintic) combined with ivermectin. In 2003 the common antibiotic doxycycline was suggested for treating elephantiasis.

Prevention: The strategy for eliminating transmission of lymphatic filariasis is mass distribution of medicines that kill the microfilariae and stop transmission of the parasite by mosquitoes in endemic communities.

SCHISTOSOMES

A genus of trematodes, *Schistosoma*, commonly known as blood-flukes and bilharzia, includes flatworms which are responsible for a highly significant parasitic infection of humans by causing the disease schistosomiasis, and are considered by the World Health Organization as the second most important parasitic disease, next only to malaria, with hundreds of millions infected worldwide.

Unlike the other trematodes, the schistosomes have separate sexes. Also, unlike the other trematodes infecting humans, the cercarial stage is infective to humans after it is released by the intermediate snail host. Schistosomes do not have a secondary intermediate host. Schistosome cercaria are fork-tailed.

Clinical Syndrome: Schistosomiasis often is a chronic illness that can damage internal organs and, in children, impair growth and cognitive development. The urinary form of schistosomiasis is associated with increased risks for bladder cancer in adults. Manifestations include: abdominal pain, cough, diarrhea, Eosinophilia, fever, fatigue, Hepatosplenomegaly (enlargement of both liver and spleen), genital sores, skin symptoms.

Diagnosis: Microscopic identification of eggs in stool or urine is the most practical method for diagnosis. The stool exam is the more common of the two.

Treatment: Praziquantel is the treatment of choice for all species of schistosomiasis. Clinical studies show that artemether, which is used as antimalarial treatment, is also active against all 3 major schistosome parasites.

Prevention: Prevention is best accomplished minimizing water exposure in endemic areas and by eliminating the water-dwelling snails that are the natural reservoir of the disease.

Leishmaniasis

Leishmaniasis is a disease caused by an intracellular protozoa parasite, and it affects as many as 12 million people worldwide, with 1.5-2 million new cases each year. The global incidence of leishmaniasis has increased in recent years because of increased international leisure- and military-related travel, human alteration of vector habitats, and concomitant factors that increase susceptibility, such as HIV infection and malnutrition.

The recent conflicts in Iraq and Afghanistan have led to approximately 2000 laboratory-confirmed cases (and at least double the number of unconfirmed cases) of cutaneous leishmaniasis and 5 laboratory-confirmed cases of visceral leishmaniasis in American soldiers alone from 2003-2008. In Colombia, the military fighting the *Fuerzas Armadas Revolucionarias de Colombia* (FARC) has seen more than 30,000 cases of leishmaniasis in the last 3 years. Of course, a significantly larger burden of diseases is borne by the local populations of these countries where *Leishmania* species are endemic. In these populations, leishmaniasis contributes greatly to morbidity and mortality.

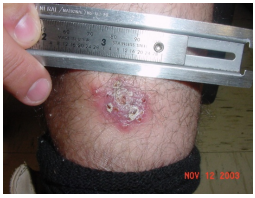
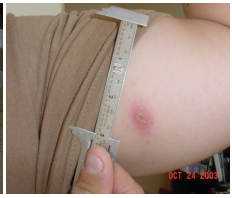
Infection is transmitted by the bite of a sandfly, which is usually one half to one third the size of a mosquito. The clinical spectrum of leishmaniasis ranges from a self-resolving cutaneous ulcer to a mutilating mucocutaneous disease and, depending on the species of *Leishmania* involved, even a lethal systemic illness. Infection with different *Leishmania* species can lead to a remarkably broad range of disease states.

Figure 1: Sandfly size comparison (Sandfly on left, *Aedes* Mosquito in the middle and *Anopheles* Mosquito on right)

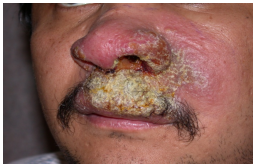
The clinical spectrum can range from insignificant pustules to fatal systemic disease. General understanding of this clinical spectrum, although once believed to be quite predictable, continues to evolve as new diagnostic techniques contribute to the elucidation of the variety of clinical manifestations of an infection with even a single species of Leishmania. The particular species associated with certain disease states originally was determined based only on clinical manifestations and location found. In current practice, molecular techniques have shown a very different parasite-to-disease association than was ever appreciated previously.

Diagnosis is often difficult because of the small size of the protozoa sequestered within macrophages of the skin, bone marrow, and reticuloendothelial system. Therapy has long been a challenge in the more severe forms of the disease and is made more difficult by the emergence of drug resistance. No effective vaccine for Leishmaniasis is available.

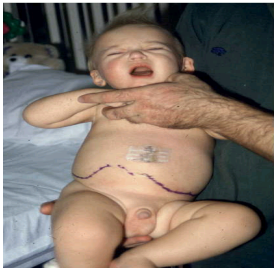
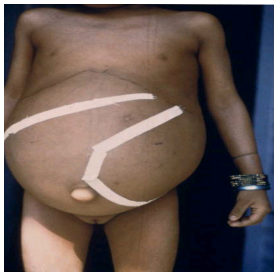
Three Clinical Manifestiations of Leishmaniasis:

Localized **cutaneous leishmaniasis**



Mucocutaneous leishmaniasis (Espundia)

Visceral leishmaniasis (deadly disease if not treated)
http://www.icp.ucl.ac.be/~opperd/parasites/images/WHO1.jpg

Figure 2: Cutaneous Leishmaniasis punch biopsy

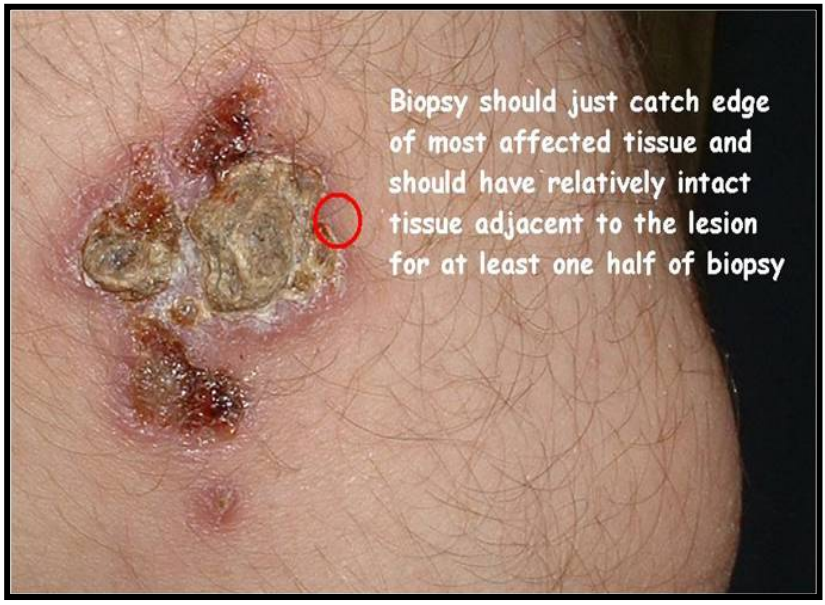


Figure 3: Where to make dermal punch for Leishmaniasis

Localized cutaneous leishmaniasis usually manifests as a nonspecific ulcer that can mimic many other infectious and noninfectious skin conditions. The vast majority of cases patients spontaneously with scarring and never come to the attention of clinicians. Even in US troops stationed in Iraq, it is currently felt, by many most closely associated with the disease and familiar with the epidemiology in the military, that less than 25% of all disease ever concerns afflicted soldiers enough to seek medical attention.

In both the localized cutaneous and mucocutaneous forms of leishmaniasis, cell-mediated immunity to the parasite is vigorous and organism density in the skin and/or mucosa is low, especially in long-standing disease (although very early in the disease large numbers of the parasites are frequently found). Therefore, growing organisms in culture can be difficult, as can finding them in pathological specimens. Malnourished individuals are at greater risk of acquiring leishmaniasis and respond less well to treatment than those with adequate nutrition.

The general consensus is that less than 5% of individuals infected by *Leishmania brasiliensis*, and a smaller percentage of individuals infected by *Leishmania panamensis* and *Leishmania guyanensis*, develop mucosal metastases several months to years after the apparent resolution of cutaneous disease. However, no rigorous studies prove this commonly accepted rate. Without treatment though, even in this small number, destruction of the oral and nasopharyngeal mucosa can be quite devastating and relentless.

Symptoms of visceral leishmaniasis can be confused with many other infectious diseases; however, in endemic areas, the typical patient has wasting and presents with massive splenomegaly, pancytopenia, hypergammaglobulinemia, and intermittent fevers (although they are less acutely ill than patients with malaria).

A typical lesion of localized cutaneous leishmaniasis begins as an inflammatory papule, which later progresses to an ulcer. This may be associated with sporotrichotic lymphatic spread. In the vast majority of cases, the ulcers heal spontaneously with scarring.

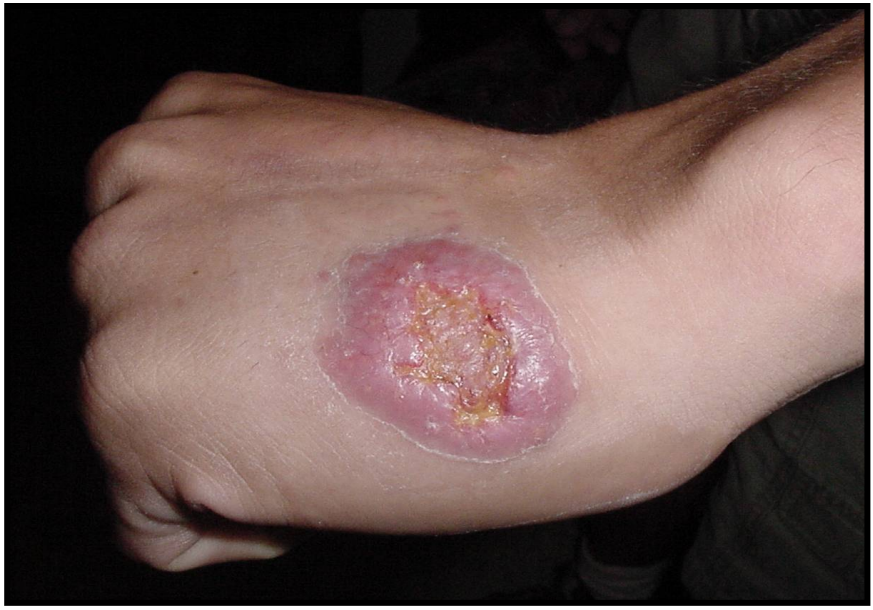


Figure 4: Cutaneous Leishmaniasis lesion

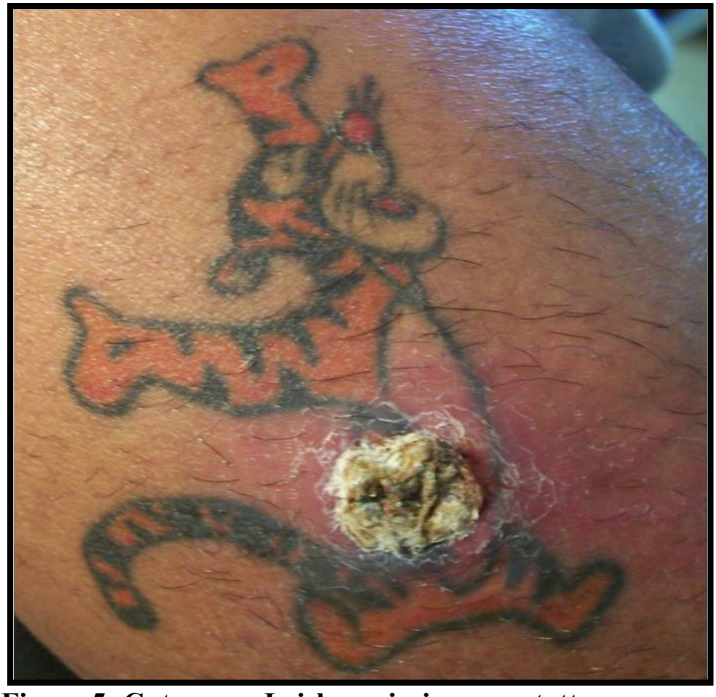


Figure 5: Cutaneous Leishmaniasis over a tattoo

Leishmaniasis Patient Information Sheet

Soldier completes Part A; Clinical provider completes Part B

PART A – SOLDIER

Patient Name: _____ **SSN:** _____ **Rank/ Service:** _____

Blood type____ **Weight** ____ **Med Allergies** _____ **Age** _____ **DOB:** _____

Unit: Company _____ **BN** _____ **BDE/BCT** _____ **DIV** _____

Date soldier arrived in Theater: _____ **in Iraq:** _____

Places/dates lived in Iraq: (e.g., *FOB Murphy, 10 Jun – 15 Jul 03*) _____

_____

Were rodents present around bivouac area? Y / N **Were dogs in the area? Y / N**

Places You Slept	# Weeks or N/A	Screens Or Windows? (Y/N)	A/C (Y/N)	Use Bednet (Always/ Sometimes/Never)	Use Repellent (Always/ Sometimes/Never)	Insect Bites Per Night? *(<5, 5-20, >20)*
Vehicle or Ground						
Tent						
Building						

Your Use of Insect Repellents	Product Was Not Available to Soldier	Product was Available to Soldier			
		Did Not Use	Used Only After Insect Bites – After how many bites? *(<5, 5-20, >20)*	Used Every Night	Used Other Times *Describe When*
Bed Net, Treated w/ Permethrin					
Bed Net w/o Permethrin					
Permethrin Treated DCUs					
DEET (green tube) on Skin					
Commercial Insect Repellent *If Yes, List in Box*					

PART B – CLINICAL PROVIDER (*Send form with slides and biopsy*)

Lesion Location & #: _____ Duration? _____

Antibiotic Treatment (type/dose/length): _____

Photos Taken? N / Y If Yes, sent to WRAIR? N / Y

Procedures Done: Scrape Biopsy: N / Y Punch Biopsy: N / Y Touch Prep: N / Y

Culture: N / Y Preserved Tissue: N / Y PCR: N / Y

Date Eval: _____ MTF: _____ POC: _____ Phone: _____

E-mail(POC): _____

Clinician Name E-mail (Provider): _____

(stamp) ==========

Results: (POS / NEG) _____

Notes: _____

For questions regarding Leishmaniasis, contact the Leish Diagnostic Lab (peter.weina@us.army.mil)

version 12Apr04

Transport or Shipping of Parasites and Tissue

1. Procedures

A. General Regulations to follow

1) Note that there is a restrictive DOD Directive on the shipment of DOD hazardous materials (guidance for this directive is 49 CFR 173).

2) Only offer for transportation shipments of hazardous materials, including biomedical materials after approval by a DOD certifying official.

3) The DOD certifying official must be approved by the commander in writing after receiving appropriate training (i.e. Transport of Biomedical Materials Course from USACHPPM).

4) Ensure any packages are certified by the DOD certifying official before shipment. DO NOT package or ship any biomedical material without certification.

B. First, determine whether the sample to be shipped is considered an infectious substance or a diagnostic specimen:

1) Diagnostic specimen: Unknown whether contents are infectious to humans, or shipment for identification.

a) Make shipment in a plain box, removing any biohazard labels.

b) Do not use an airbill for CONUS diagnostic shipments. See local logistician to enter shipment information into shipment computer.

c) For OCONUS diagnostic shipments, use the following instructions.

(1) Mark To: and From: with appropriate addresses on the side of box.

(2) Place both diagnostic and directional arrow stickers on the side of box.

(3) Ensure to over pack box with absorbent material.

(4) Ship only a MAXIMUM of 50mls of media per package.

(5) Place FED EX sleeve onto top of box that contains airbill and CDC permit.

(6) Follow these instructions for airbill:

-Fill out To: Receiver's information.

-Fill out From: Lab Director information.

-Ensure account information is correct.

-Check FED EX Priority overnight (4A).

-Check "Yes" for dangerous goods (6).

-Weigh box and note on airbill.

-Make copy of airbill to track pack.

(7) Sign airbill at release signature block. Follow common procedures for completion.

2) Infectious substance: Known to contain material infectious to humans and concentrated.

a) Make shipment with a 4G/Class 6.2 Isocontainer from Casing Corp.

b) Ensure proper address labels are affixed to box.

(1) From: Physician and facility information (receiving).

(2) To: Lab Director information

c) Enter the Name of responsible party (at top and bottom of box) such as "Name and phone number".

d) Ensure proper labels attached to side of box:

(1) Infectious substance (6) sticker

(2) UN2814 Infectious substance [fill out "Liquid Leishmania (amount in mls)"]

(3) Etiologic agents / biomedical material (6) sticker

e) Ship only a MAXIMUM of 50mls of media per package.

f) Place FED EX sleeve on top of box that contains airbill, CDC permit, and Shipment of dangerous goods form

g) Follow these instructions for airbill:

(1) Fill out To: Receiver's information

(2) Fill out From: Lab Director's information.

(3) Ensure account information is correct.

(4) Check FED EX Priority overnight (4A).

(5) Check “Yes” for dangerous goods (6).

(6) Weigh box and note on airbill.

(7) Make copy of airbill to track package.

h) Sign airbill at release signature block.

C. Instructions for declaration of dangerous goods.

1) Write in “Shipper”: Lab Director’s information.

2) Write in “Consignee”: Receiver’s information.

3) Blot out Cargo Aircraft only and radioactive boxes.

4) Type everything under nature and quantity of dangerous goods in capital letters.

a) Under proper shipping name: “INFECTIOUS SUBSTANCE AFFECTING HUMANS (LIQUID) LEISHMANIA”

b) Class or division: “6.2”

c) UN or ID No.: “UN2814”

d) Under quantity and type of packaging: 4G, # of flasks contained, amount of total media in mls.

e) Packing instructions: “602”

f) Under additional handling information: “FOLLOW DIRECTIONS ON CONTAINER PRIOR ARRANGEMENTS AS REQUIRED BY THE IATA DANGEROUS GOODS REGULATION 1.3.3.1 HAVE BEEN MADE IN ACCORDANCE WITH ICAO AND IATA REGULATIONS”

g) Under emergency Telephone # place local contact, such as: 301-319-9956 / 9497, pager 301 369-5413, fax 301-319-7360

h) Fill out signature block with sender’s information.

i) Sign signature block.

D. Common Procedures

1) Wrap in brown paper and tape up final package. Attach the final FED EX sleeve to the top of box.

2) Ensure the following:

a) Sign release signature.

b) Make copy of airbill to track package.

c) Give top copy of airbill goes to comptroller for accountability.

d) Bring box to shipping section.

e) Place box in FED EX shipping area.

f) Log in / fill out shipment notice form (POC "name and telephone").

Slide Preparation and Staining

1. Materials and Equipment

A. Slide preparation

1) Slides

2) Cover slips

3) Methanol

4) Plastic disposable pipettes

5) Cytospin

6) Timer

7) Cytospin holder

8) Pencil, marker

9) PBSS

10) Tissue

11) Disposable alcohol wipes

12) Small Avery labels

B. Staining

1) Methanol

2) Giemsa / Diff-Quick Solutions 1 & 2

3) Forceps

4) Copeland jars

5) Water (Analytical type I reagent grade water)

6) Water (Sigma® sterile water)

7) Microscope

8) Paper towels

9) Permount

10) Immersion oil

11) Slide box

12) Filters (0.22μm)

2. Procedures

A. Slide Preparation

1) Direct Slide Preparation

a) Clean slide with alcohol wipe. With forceps grasp the tissue and make impression on the slide by either making a dabbing or circular motion on the slides with the tissue (let it dry).

b) Place a drop of mixture on the slide and smear the slide, if a tissue/PBSS mixture. Air dry slide before use.

2) Using Cytospin

a) Put 2 - 3 drops of ground tissue on each slide and place in separate cytospin holder. Ensure an equal amount is placed in on each slide.

b) Spin for 3 min @ 800 rpm. Repeat if necessary.

c) Remove slide from holder and let dry for staining.

B. Staining

1) Using Diff-Quik

a) Fix slide with methanol. Methanol should cover the entire slide area. Let slide air dry under hood.

b) Filter stain prior to use.

c) Get 4 Copeland jars ready for staining by filling the jars 3/4 full with the following:

(1) Jar 1: Diff-Quick I

(2) Jar 2: type I water

(3) Jar 3: Diff-Quik II

(4) Jar 4: type I water.

d) Grasp the slide and submerge it into Diff-Quik solution I for 45 seconds using forceps.

e) Remove slide and wash gently in jar 2 type I water.

f) Submerge slowly in type I water 3 times.

g) Damp slide edge on paper towel.

h) Submerge slide into Diff-Quik solution II for 45 seconds.

i) Remove slide and wash gently in jar 4, type I water.

j) Submerge slowly in type I water 3 times.

k) Let slide air dry.

2) Giemsa Stain

a) Fix slide in methanol and let air dry.

b) Prepare a 30% concentration of Giemsa in Sigma® water using 50 ml conical tube. Filter 30% Giemsa solution prior to use.

c) Pour 30% Giemsa solution on slides and leave slide flooded with solution for 30 minutes

d) Wash slide thoroughly with type I water.

e) Let slide air dry under hood.

f) Read.

Slide Reading

1. Materials and Equipment

A. Immersion oil

B. Cover slip

C. Smeared and stained slide

D. Microscope

E. Permount with 10% xylem

F. Control positive slide

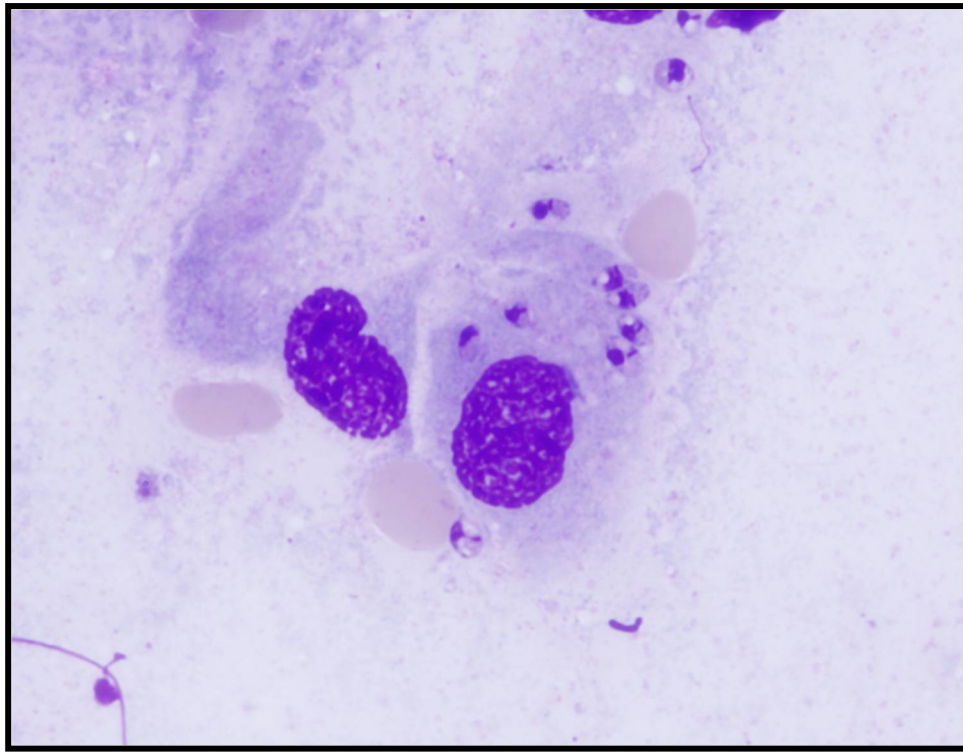
2. Procedures

A. Place slide on microscope after airdrying under hood.

B. Use an oil immersion lens in order to view the slides

C. Complete a comprehensive review of the slide in order to visualize any amastigotes (Leishmania parasites which lack a tail) on the slide.

Figure 6: Amastigotes on the Giemsa stained slide



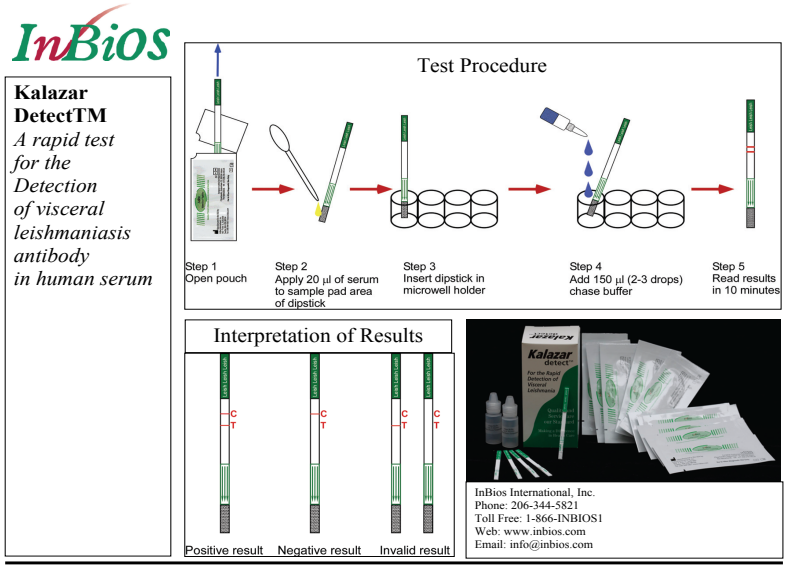
D. Use a positive control slide if necessary to confirm visualization of amastigotes.

E. Write a report with the technician's impressions on the slide quality and slide result. Put this with the patient file.

F. Label the slides and slide box with the local control number, date, patient name, and the technician's initials.

G. Bring the slides to the Lab Director for confirmation.

rK39 Serum Test Strip



The InBios slide with the Kalazar DetectTM test

InBios International, Inc.

Phone: 206-344-5821 Toll Free: 1-866-INBIOS1

Web: www.inbios.com Email: info@inbios.com

1. Procedures

A. Follow product insert from INBIOS for rK39 strip test for visceral leishmaniasis.

B. Record results in notes section of LDL Accession spreadsheet and notify Lab Director of results.

C. Follow these procedures for archiving patient serum

1) Fully aliquot positive serums into as many 1.8ml cryotubes are needed and labeled by local control number.

2) Aliquot negative serums once and properly label by local control number.

3) Store all serum aliquots in chronological order.

InBiOS International

Kalazar Detect™ Rapid Test

for the Detection of Visceral Leishmaniasis Antibody in Human Serum

Intended Use

The *Kalazar Detect*™ Test for Visceral Leishmaniasis (VL) is a rapid immunochromatographic strip assay for the qualitative detection of antibodies to members of *L. donovani* in human serum. The assay is for the aid in the presumptive diagnosis of VL. This test strip is intended for professional *in vitro* diagnostic use only. It is not intended for use in blood donor centers or blood component manufacturers.

Summary and Explanation

VL is a severe disease with high mortality, endemic in 88 countries including 17 developed nations (1,2). A serious problem in much of the world including Brazil, China, East Africa, India and areas of the Middle East, leishmaniasis is also endemic in the Mediterranean region including southern France, Italy, Greece, Spain, Portugal and Northern Africa. In areas where leishmaniasis is endemic, recent migration patterns of people, vectors (sandfly) and reservoirs (dogs) have led to the urbanization of VL (3). In Southern Europe, VL has become the leading opportunistic infection in AIDS patients (4,13).

VL is caused by members of the *Leishmania donovani* complex and canines have been identified as the major reservoir for transmission (5-8). Serodiagnosis has been widely utilized to establish infection because antileishmanial antibody titers are high during acute disease. The preferred method of diagnosis in a laboratory situation is by ELISA, although fluorescent antibody (IFAT) or direct agglutination tests (DAT), both utilizing whole parasites, are still widely used (9-11). These tests are highly cross-reactive with trypanosomes and mycobacteria. In addition, the whole parasite preparations used are unstable and variable in quality. This rapid assay is for the qualitative determination of antibodies to a recombinant antigen specific for Visceral Leishmaniasis (12) caused by parasite members of the *L. donovani* complex.

Principle

The *Kalazar Detect*™ Test for VL is a qualitative, membrane based immunoassay for the detection of antibodies to Visceral Leishmaniasis in human serum. The membrane is pre-coated with rK39 on the test line region and chicken anti-protein A on the control line region. During testing, the serum sample reacts with the dye conjugate (protein A-colloidal gold conjugate) which has been pre-coated in the test device. The mixture then migrates upward on the membrane chromatographically by capillary action to react with recombinant VL antigen on the membrane and generates a red line. Presence of this red line indicates a positive result, while its absence indicates a negative result. Regardless of the presence of antibody to rK39, as the mixture continues to migrate across the membrane to the immobilized chicken anti-protein A region, a red line at the control line region will always appear. The presence of this red line serves as verification for sufficient sample volume and proper flow and as a control for the reagents.

Precautions

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- Handle all sera and kits used as if they contain infectious agents. Observe established precautions against microbiological hazards while performing all procedures and follow the standard procedures for proper disposal of sera and used kits.
- Wear protective clothing, eye protection and disposable gloves while performing the assay. Wash hands thoroughly when finished.
- Avoid all contact between hands and eyes or mucous membranes during testing.
- Do not eat, drink or smoke in the area where the sera and kits are handled.
- Chase Buffer contains a preservative; avoid all possible contact with skin and mucous membranes.

Storage

The sealed pouch or vial containing the test strip is designed to be stored at room temperature (20°C-28°C) for the duration of its shelf life. The bottle containing the Chase Buffer is designed to be stored at room temperature for the duration of its shelf life. Exposure to temperatures over 30°C can impact the performance of the test and should be minimized. The strips should not be frozen. The test should be used within 1 hour after removal from the pouch or vial to prevent exposure to humidity.

Sera Collection

- Human serum should be tested with this test strip. Whole blood should not be used with this test as it may affect ones ability to read the test line correctly due to excessive background. Dilutions of serum in buffer cannot be tested directly. Positive serum can be diluted with disease negative sera.
- Remove the serum from the clot of red cells as soon as possible to avoid hemolysis.
- Test should be performed as soon as possible after sera collection. Do not leave sera at room temperature for prolonged periods. Sera can be refrigerated at 2-8°C up to 3 days. Otherwise sera should be stored below 20°C.
- Bring sera to room temperature prior to testing. The frozen sera must be completely thawed prior to testing. Sera should not be repeatedly frozen and thawed.
- If sera are to be shipped, they should be packed in compliance with Federal Regulations covering transportation of infectious agents.

Kit Contents

Kalazar Detect test strip's membrane is pre-coated with a recombinant rK39 on the test line region and chicken anti-protein A on the control line region.
The Kit contains the following:

1. Twenty-five (25) individually pouched Test Strips or twenty-five (25) test strips in a vial with desiccant in the cap.
2. One (1) vial of Chase Buffer solution.

Test Procedure

1. Allow the sera to reach room temperature prior to testing.
2. Remove the *Kalazar Detect*™ Test for VL from the foil pouch or vial.
3. Add 20 µl of sera to the test strip in the area beneath the arrow.
4. Place the test strip into a test tube, or well of a 96 well tissue culture plate so that the end of the strip is facing downward as indicated by the arrows on the strip.
5. Add 2-3 drops (150 µl) of the Chase Buffer solution provided with this test kit.
6. Read the results in 10 minutes. It is significant that the background is clear before reading the test, especially when samples have low titer of anti-Leishmanial antibody, and only a weak band appears in the test region (T). Results interpreted after 10 minutes can be misleading.

Note: Do not test this product with the Chase Buffer solution alone. 20 µl of human serum must be added first.
Note: If migration of the gold is not observed within 10-15 seconds after the addition of chase buffer, lightly press on sample tape region of dipstick until migration of gold is observed.

Interpretation of Results

A Positive Result

The test is positive when a control line and test line appear in the test area as shown in Figure 1. A positive result indicates that the Kalazar Detect dipstick detected antibodies to members of *L. donovani* complex. A faint line is considered a positive result. As a guide for interpretation, the red color in the test region will vary depending on the concentration of anti-Leishmanial antibodies present. The test line for "weakly positive" sera samples may show results between a weak positive red line to a faintly red, almost white background. ("Weakly positive" samples are those with low affinity or low titer antibodies against the recombinant test antigen.)

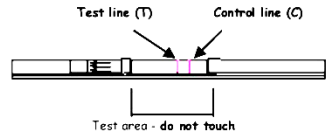


Figure 1

A Negative Result

The test is negative when only the control line appears. A negative result indicates that the Kalazar Detect dipstick did not detect antibodies to members of *L.donovani* complex. No test line is present as in Figure 2.

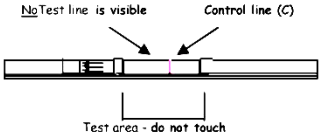


Figure 2

An Invalid Result

No lines appear at either the control or test line areas. The test is also invalid if no control line appears, but a test line is seen. It is recommended to retest using a new *Kalazar Detect*™ Test for VL and fresh serum.

Note: The red color in the test region will vary depending on the concentration of anti-Leishmanial antibodies present. However, neither the quantitative value nor the rate of increase in antibodies can be determined by this qualitative test.

Expected Value:

In endemic areas, the sensitivity of the Kalazar Detect test is 90% or better. The specificity of the test may vary with geographic location. For example, in India thirteen out of 104 healthy controls showed positive reactivity with the Kalazar Detect Test.

Performance Characteristics

Reproducibility Study:

The reproducibility of the Kalazar Detect test strip was evaluated at 3 sites using a panel of confirmed VL sera. Positive, low/weak and normal serum samples were used. The samples were coded and tested at each site in triplicate for 3 consecutive days. . The results indicate that for each day, the technician scored the test the same. Once the samples were decoded, the reading was in line with the ELISA titer. This data indicates that the reproducibility of the Kalazar Detect Test strip is excellent.

Interference Studies:

Indian Study: Patients with neoplastic disease, viral infection, chronic bronchitis, amebic liver abscess, idiopathic thrombocytopenic purpura, rheumatic heart disease, myelodysplastic syndrome, myoclonus, leprosy, tuberculosis, syphilis and malaria were tested with the Kalazar Detect test strip for the presence of Leishmania. Only one patient with malaria produced a false positive result. All other patients tested negative.

Brazilian Study: Sera from patients with malaria, chagas, tuberculosis, cutaneous leishmaniasis and Hansen disease were tested with the Kalazar Detect test strip for the presence of visceral leishmaniasis. All patient sera tested negative for Leishmania.

Field Studies:

The *Kalazar Detect*™ test for VL was field tested at 2 sites. The table below summarizes the results of these studies.

Site 1: Brazilian Study: Kalazar Detect Test Compared to Microscopy

		+	-	
Kalazar Detect	+	115	0	
	-	13	59	
		128	59	187
Sensitivity	89.844		**Specificity**	100
Std. Error	2.67			0
95% CI	(82.936, 94.263)		(92.384, 100)	

Site 2: Indian Study: Kalazar Detect Test Compared to Microscopy

		+	-	
Kalazar Detect	+	225	14	
	-	0	190	
		225	204	429
Sensitivity	100		**Specificity**	93.137
Std. Error	0			1.77
95% CI	(97.908,100)		(88.517, 96.054)	

Note: Site 2 had a high prevalence of VL patients.

Limitations

- This test will only indicate the presence of antibodies to the recombinant test antigen rK39 in patients with Visceral Leishmaniasis and should not be used as the sole criterion for the diagnosis of Leishmaniasis. This test alone must not be used for any clinical treatment decision. As with all diagnostic tests, all results must be considered with other clinical information available to the doctor.
- If the result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is recommended. A negative result does not preclude the possibility of Leishmaniasis.
- A false positive result may occur. Confirmatory testing (such as by culture) is advised especially in cases where no symptoms exist.
- Do not use serum samples containing any glycerol or other viscous materials. This will decrease the sensitivity of the assay.
- Persons with advanced HIV infection or other immunocompromised diseases frequently have low or undetectable anti-Leishmanial antibodies.
- This test may yield false positive results with samples from patients having malaria.
- The performance of this test has not been evaluated with L. infantum.
- Certain Rheumatoid Factor (RF) sera may produce false positive results when Kalazar Detect is used.

Reference

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IVD

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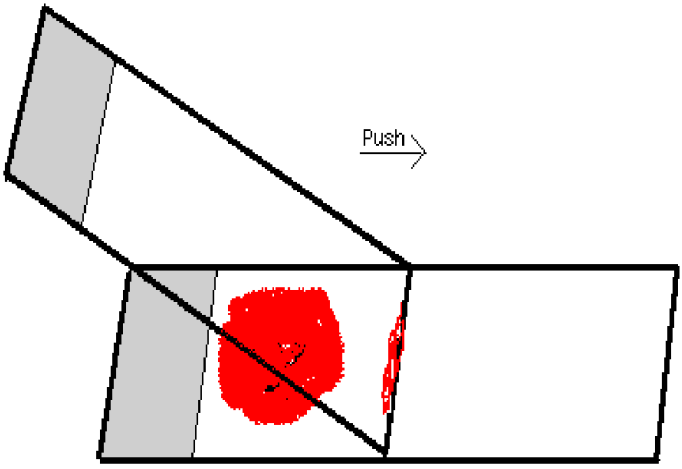
CE

Malaria Diagnostics

Laboratory Procedures

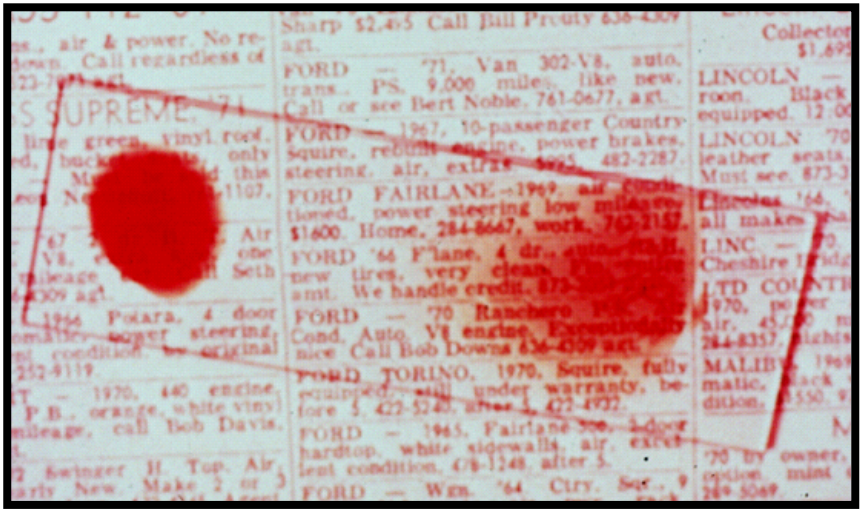
A. Making a thick and thin smear. (Instructions modified from references 1,2, and 4).

1. Label two slides with your name and the date. Use a pencil, not a pen.
2. Wipe the ball of your middle finger with an alcohol pad.
3. Dry the finger with a Kimwipe.
4. Using a sterile lancet, puncture the ball of the finger.
5. Apply gentle pressure to the finger and express the first drop of blood; wipe it away with a Kimwipe.
6. Working quickly and handling the slides only by the edges, collect the blood as follows:
7. Apply gentle pressure to the finger and collect a single small drop of blood about 3 mm in diameter • on the middle of the slide. This is for the thin film.
8. Apply further gentle pressure to express more blood, and collect two or three larger drops on the slide, about 1 cm away from the drop intended for the thin film. Wipe the remaining blood off the finger with a Kimwipe.



9. Use another clean slide as a "spreader", and with the slide with blood resting on a flat, firm surface, touch the edge of a clean slide at a 45-degree angle to the slide with blood just in front of the single drop of blood for the thin film. Slowly draw back the clean slide while securing the sample slide with the forefingers of the other hand. Barely touch the drop of blood and, as the blood spreads laterally along the edge of the clean slide, rapidly and firmly push the clean slide forward, making sure to maintain even contact at all times between the spreader and the surface of the slide, and not stopping until the clean slide leaves the bloody slide.

10. **The thick film:** Handling the slides by the edges or a corner, make the blood film by using the corner of the spreader to join the drops of blood, and spread them to in a circular pattern to make an even, thick film. The circular thick film should be about 1 cm in diameter. A thick smear of the proper density is one which, if placed (wet) over newsprint, allows you to barely read the words. Make two thick smears for every patient.



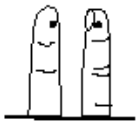
Example of thick smear (left) and thin smear (right)

11. Place the heat gun at high setting about 6-7 inches away from the slides in a rack and dry for about 15 minutes. Never make the slides too hot to touch, otherwise the red cells will be fixed and they will not lyse.
12. The CDC DPDx Web site says the following about drying: "Insufficiently dried smears (and/or smears that are too thick) can detach from the slides during staining. The risk is increased in smears made with anticoagulated blood. At room temperature, drying can take several hours; 30 minutes is the minimum; in the latter case, handle the smear very delicately during staining. You can accelerate the drying by using a fan or hair dryer (use cool setting). Protect thick smears from hot environments to prevent heat-fixing the smear." Our lab dries thick smears using a slide warmer set at 37^{o}C for 15 minutes, but slide warmers may not be available in the field. Drying procedures will need to be adjusted depending on the heat and humidity of the environment.
13. From reference 2: "Flies, ants, cockroaches and other insects eat the wet or drying blood and damage the films. Slides should be covered during drying and then stored overnight in an airtight box or desiccator charged with silica gel."

FIGURE A-1. Blood collection for thin or thick blood film

1

Select the finger to puncture (usually the third or fourth finger).



2

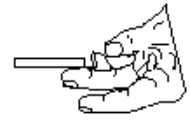
Puncture the side of the ball of the finger. Do not make the puncture too close to the nail bed.

3

If the blood does not well up from the puncture, gently squeeze the finger.

4

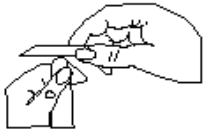
Always grasp the slide by its edges.



5

To control the size of the blood drop on the slide, touch the finger to the slide from below.

FIGURE A-2. Preparation of a thin and thick blood film on the same slide



1

Touch the blood drop with a clean slide.

2

Using the corner of another slide, spread the blood drop into the shape of a circle or square of ~1 cm^3.

3

Gently squeeze the patient's finger again, and touch the edge of a clean slide to the newly formed blood drop.

4

Take this slide and hold the edge that has the blood drop at an ~45° angle against the surface of the first slide. Wait until the blood completely spreads along the edge of the second slide.

5

While holding the second slide at the same angle, rapidly and smoothly push the slide forward.

6

Write the identification number on the slide. Wait until the thick film is completely dry before staining it.

B. Staining the smears (rapid method)

1. Mix 1 ml of Giemsa stain with 9 ml of Giemsa buffer to make a 10% solution.
2. Place the slides on a staining rack face up and separated from one another.
3. Using a transfer pipette, put stain on top of the smears to cover them completely.
4. Incubate at room temperature for 10 minutes.
5. Using a wash bottle, rinse the stain off the slides and into the sink with tap water. Squirt the water onto the label portion of the slide and allow it to wash the stain off the slides. Do not pour the stain off the slides, or it will leave a green scum behind.
6. Put the slides in a drying rack and place the rack next to a fan to dry.
7. When the slides are dry, put them into a box or slide holder and bring them to a microscope.

Note: Our standard staining method is to use 4% Giemsa stain for 45 minutes. The 10-minute rapid method is said to result in more artifacts, though we find it to be satisfactory.

B. Examining the slides under the microscope (modified from reference 3)

1. Place the slide on the microscope stage with the specimen directly over the lamp.
2. Gently place a drop of immersion oil on top of the specimen (or the cover slip).
3. Make sure the stage is low enough so that you can rotate the oil immersion objective into the light path without having it hit the slide or the oil.

4. Rotate the nosepiece so that the oil immersion objective (usually 100x) is in the light path. Avoid getting oil on any of the other objectives, which are not designed to be used with oil. If by mistake you get oil on another objective, wipe it off immediately using lens paper (NOT a Kimwipe, paper towel or tissue).
5. While looking at the microscope from the front or the side (not through the observation eyepieces), slowly raise the stage until the front of the oil immersion objective makes contact with the oil drop. You will see a sudden flash of light when contact is made.
6. Now, using the fine adjustment knob only, with your left hand, continue to raise the stage until the specimen comes into focus. Meanwhile, with your right hand, use one of the stage control knobs to move the slide rapidly back and forth. This will help you to find the point at which the specimen is in focus.

Procedure to focus the microscope for both eyes

1. While looking through the eyepieces of the binocular observation tubes, grasp the binocular tubes with both hands and bring the tubes closer together (or further apart) to fuse the circles of light into one circle. This sets the interpupillary distance for YOUR eyes. If the viewing tubes have a scale for this setting, memorize the number so that you can easily return to the setting the next time.
2. Place a specimen on the stage. Using your RIGHT eye, and your right eye only, bring the object into focus.
3. Now using your left eye and your left eye only, WITHOUT touching the focusing knobs, rotate the knurled ring on the left eyepiece tube to bring the object into focus for your left eye. This procedure adjusts for differences in acuity between your left and right eyes.

Blood slides that are well-stained with Giemsa stain will have both red and blue objects. This indicates that the two colored components of the stain, methylene blue and eosin, are visible.

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Malaria Pictures and Diagrams

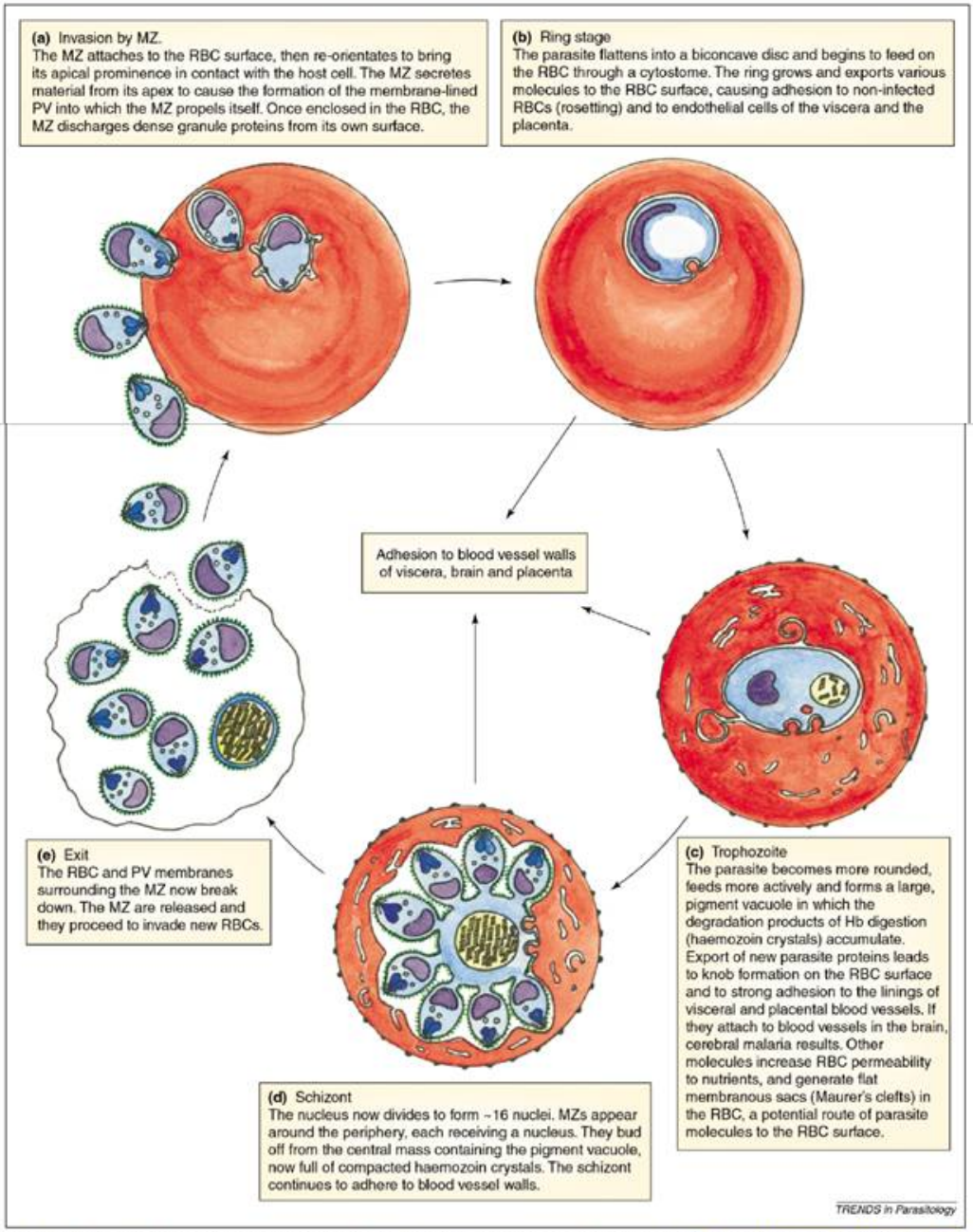
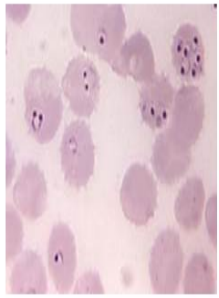


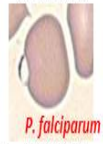
Fig. 2. The main stages of the asexual erythrocytic cycle of *Plasmodium falciparum*. For an animated version: see http://archive.bmn.com/supp/part/bannister.html. Abbreviations: Hb, haemoglobin; MZ, merozoite; PV, parasitophorous vacuole; RBC, red blood cell. See Ref. [29] for further details and illustrations.

Features to Look for in Slides for Tropical Medicine Course

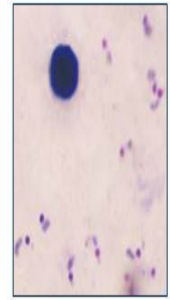


P. falciparum multiply-infected cells double chromatin dots, and headphones

P. falciparum marginal form



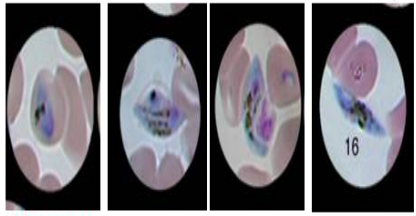
P. falciparum blister form



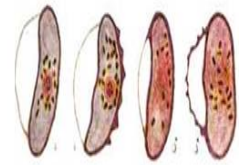
P. falciparum rings in thick film, showing a white blood cell

P. falciparum growing trophozoite with Maurer's clefts (red dots). This stage is seldom seen in peripheral blood.

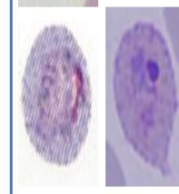
P. ovale comet form in thin film



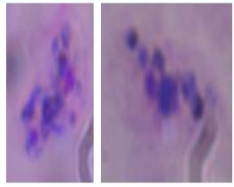
P. falciparum immature gametocytes, which are not normally seen in peripheral blood, shown in thin film. Some of the course slides show these forms.



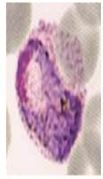
P. falciparum mature gametocytes in thin film. Note the rounded ends, compared with the pointed ends of immature gametocytes. Left, ♀, right ♂.



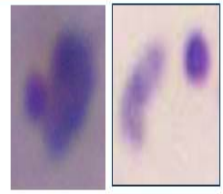
P. ovale in thin film showing fimbriae (top) and James' dots

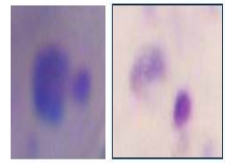


Above: ***P. vivax*** trophozoites in thick film showing broken cytoplasm



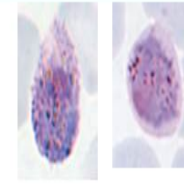
Left: ***P. vivax*** trophozoite in thin film showing Schüffner's dots

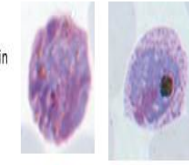




Above left: ***P. vivax*** rings compared to *P. falciparum* rings (above right) in thick film

Daisy-head schizonts of ***P. knowlesi*** (above) and ***P. malariae*** (below) in thin film

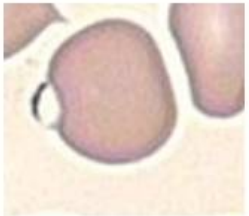




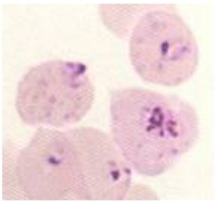
P. knowlesi gametocytes

P. falciparum

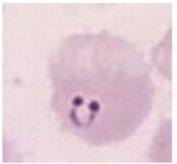
P. falciparum marginal Form (1)



P. falciparum blister form (1)



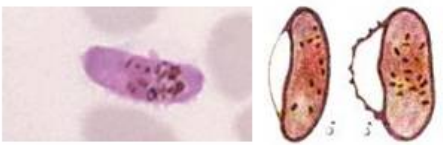
Maurer's clefts in *P. falciparum* (2)



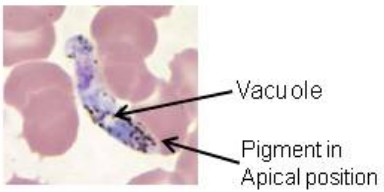
P. falciparum headphone-shaped ring (3)

P. falciparum Several rings in one red cell (4)

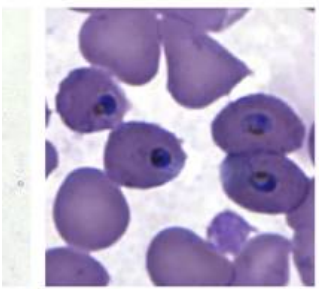
P. falciparum macrogametocytes (left, ref. 3; right, ref. 5)



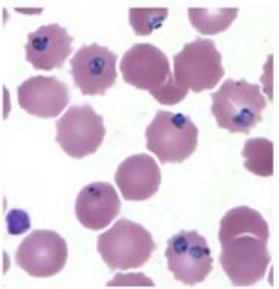
P. falciparum microgametocytes (left, ref. 3; right, ref. 5)



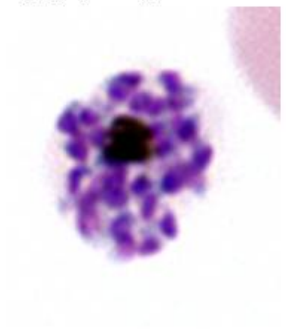
P. vivax ookinete can be confused with *P. falciparum* gametocyte (6)



Mature, compact trophozoites in a thin blood smear.



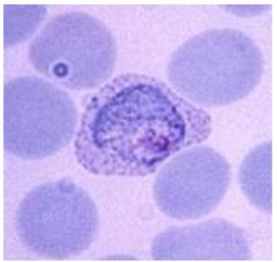
Compact trophozoites in a thin blood smear.



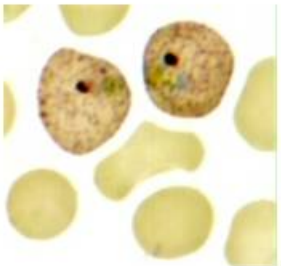
P. falciparum trophozoites and schizonts are rarely seen in peripheral blood except in a very heavy infection (left-to-right: ref. 6, ref. 6, ref. 7)

Some *P. falciparum* gametocytes have a pink attachment called a Garnham's body. (8)

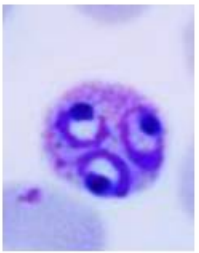
P. vivax



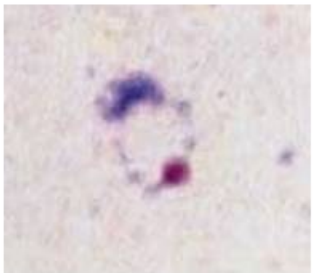
P. vivax ameboid trophozoite, Schüffner's Dots, enlarged infected cells (4)



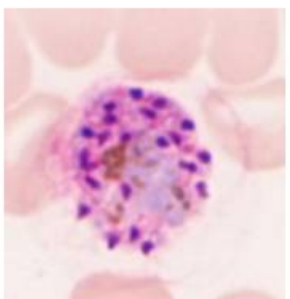
P. vivax with Schüffner's dots, Infected cells are larger than uninfected cells. (5)



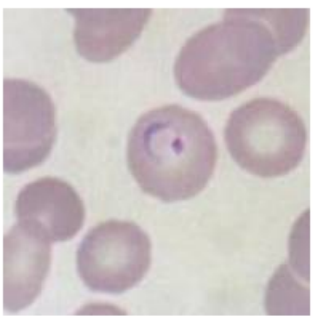
Triply-infected *P. vivax* ring (multiple infection is not restricted to *P. falciparum)* (4)



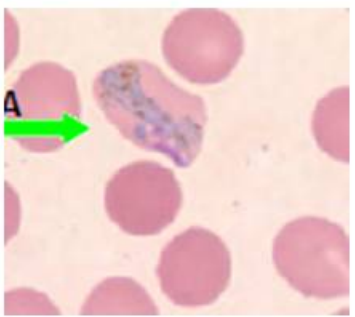
P. vivax rings in thick film showing thin filaments of cytoplasm (3)

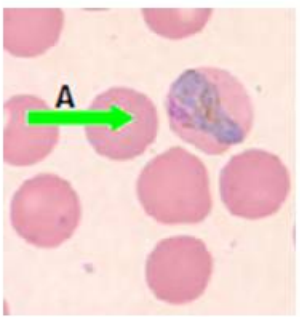


P. vivax schizont with about 21 merozoites and clumped pigment (15)



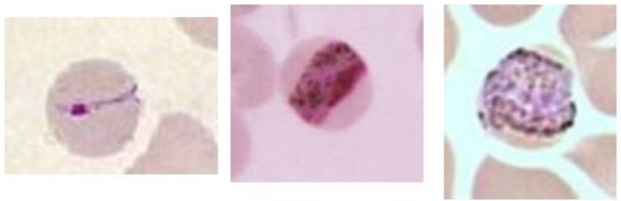
P. vivax signet ring with enlarged infected cell (2)



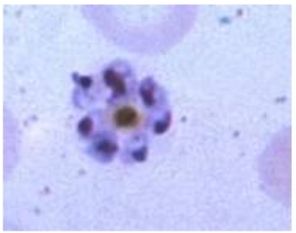


Band forms are usually found in *P. malariae*, but these band forms are identified as *P. vivax* rather than *P. malariae* by their distorted shapes and large size (12)

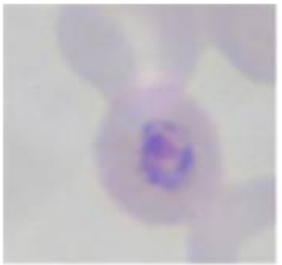
P. malariae



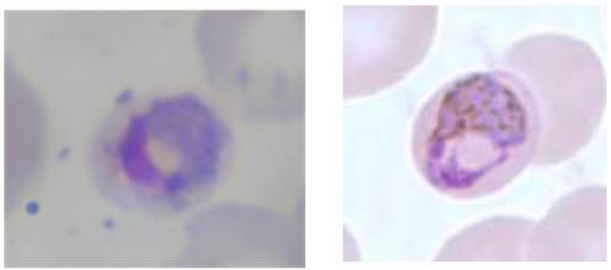
P. malariae band forms (from left to right, references 2, 4, and 2)



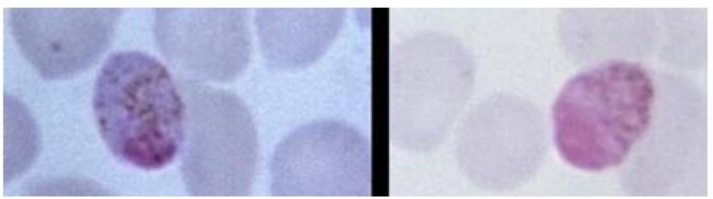
P. malariae daisy-head (rosette) schizont; similar schizonts can be seen with *P. ovale* and *P. knowlesi* (4)



P. malariae bird's-eye trophozoite; these can sometimes be seen with *P. falciparum, P. vivax,* and *P. ovale.* (10)



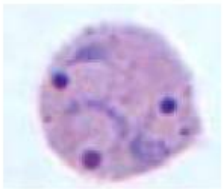
P. malariae basket trophozoites (left, ref. 10; right, ref. 7)



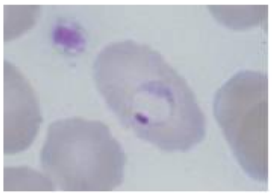
P. malariae microgametocyte (left) and macrogametocyte (right) (ref. 4)

Intensive staining or staining at a pH of about 7.5 occasionally produces pink dots called Ziemann's stippling. They are not seen with standard staining procedures. For a picture of Ziemann's stippling, see "Dots and Stippling" page.

P. ovale

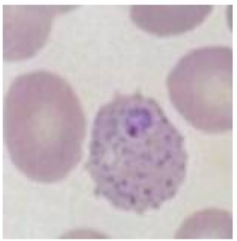


P. ovale with multiply-infected RBC (7)

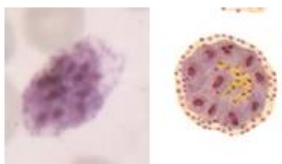


P. ovale oval ring (13)

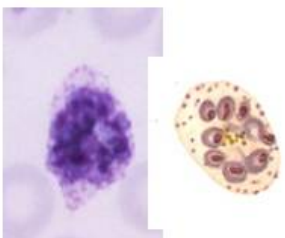
P. ovale comet forms (2)



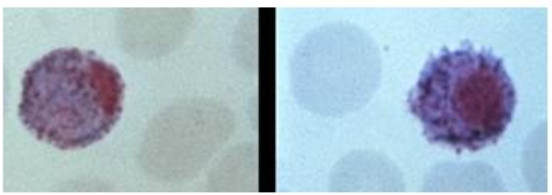
P. ovale with fimbriated edge and prominent James' Dots (2)



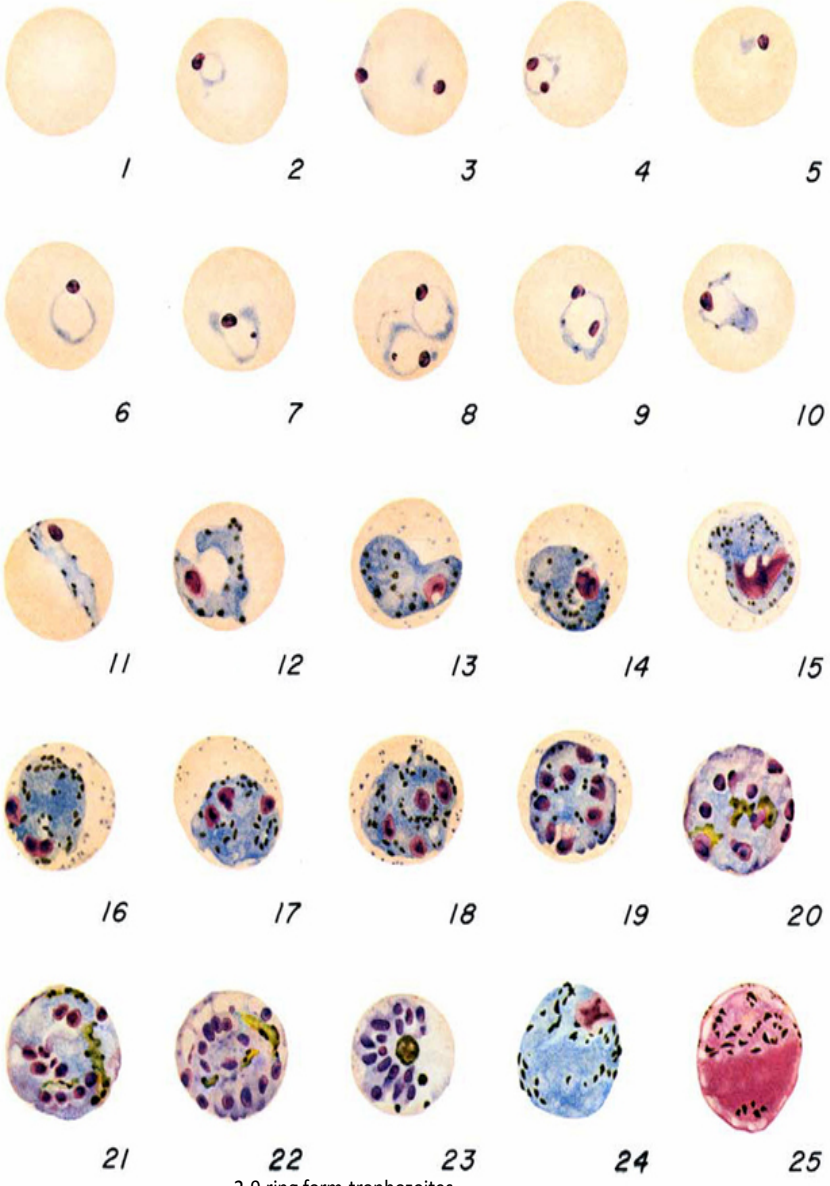
P. ovale immature schizonts (left, ref. 4; right, ref. 6)



P. ovale mature schizonts; some show "rosette" form like those of *P. malariae*, but the infected cells are usually larger than uninfected cells and may show the distorted shapes characteristic of *P. ovale*. (left, ref. 4; right, ref. 6)



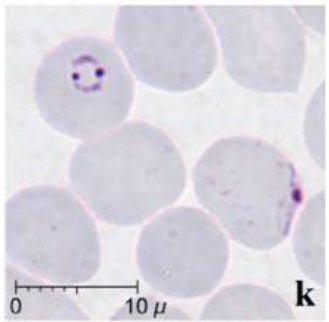
P. ovale microgametocyte (left), and macrogametocyte, right (4)



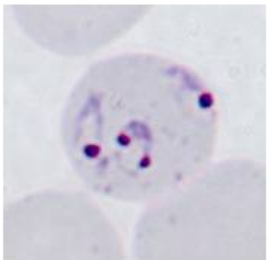
2-9 ring form trophozoites
10-12 developing trophozoites
13-15 mature trophozoites
16-23 schizonts
24 female gametocyte
25 male gametocytes

PLASMODIUM KNOWLESI

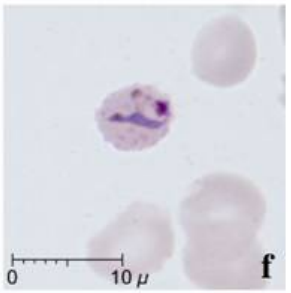
P. knowlesi



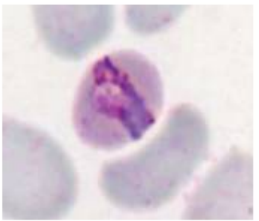
P. knowlesi with double chromatin dot and appliqué or marginal form. Both forms are also found in *P. falciparum*. (14)



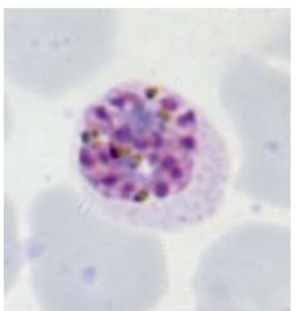
RBC with three *P. knowlesi* rings (14)



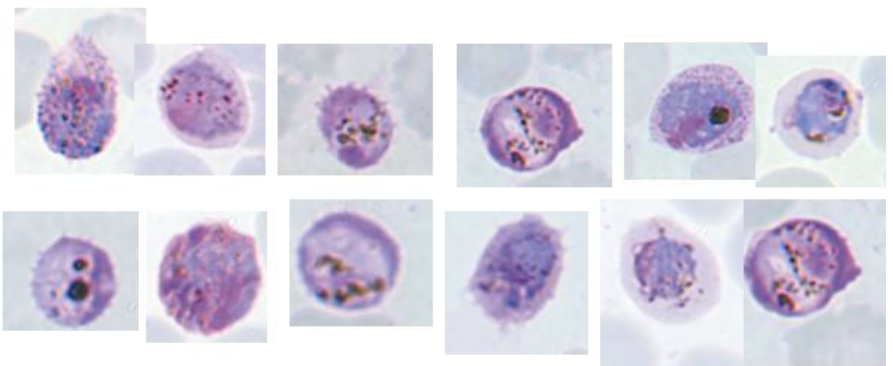
P. knowlesi trophozoite with ameboid cytoplasm and stippling. Faint stippling (Sinton and Mulligan's Stippling) is found in some erythrocytes infected with mature trophozoite and schizont stages (14)



P. knowlesi band form (16)

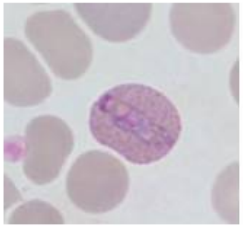


P. knowlesi daisy-head or rosette schizont with about 16 merozoites. *P. malariae* schizonts have a similar appearance, but they have 6-12 merozoites (14)

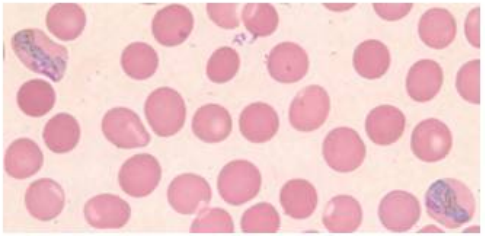


Gametocytes of *P. knowlesi* (14)

Surprising Forms



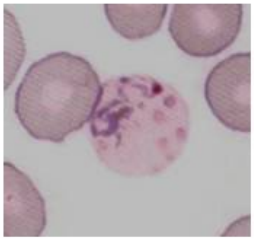
P. ovale



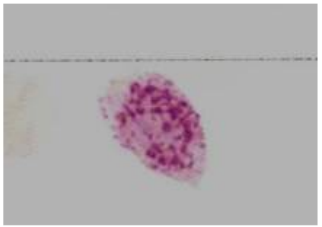
P. vivax

P. falciparum

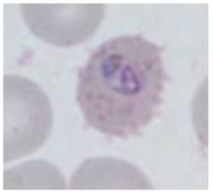
Band forms are usually associated with *P. malariae* and *P. knowlesi*. However, they are seen with *P. falciparum* if blood has been left standing, and they are also seen with other species of *Plasmodium*. Note that in the picture on the left, the band form host cell is enlarged and stippled, which is characteristic of P. ovale, and that in the picture in the middle, the infected cells are enlarged and distorted, which is Characteristic of P. vivax. The identification of *P. falciparum* in the right-hand picture was based on other parasites in the slide. It is important to observe more than one parasite before identifying the species of parasite, and to use criteria more definitive than band forms. (7)



The ameboid form of the cytoplasm and the enlarged red cell suggest the presence of *P. vivax*, but the Maurer's clefts show that the species is *P. falciparum* (3)

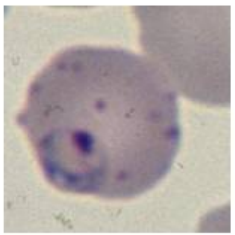


The oval shape and fimbriated edge of this cell suggest P. ovale, but the number of nuclei, >16, indicates *P. vivax* (3).

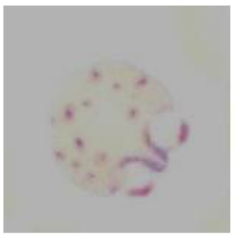


P. falciparum-infected cell with fimbriae. Fimbriae are usually associated with *P. ovale*. However, you should not base your identification on this feature alone. (7)

Dots and Stippling

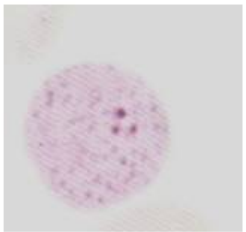


P. falciparum with Maurer's clefts (13)

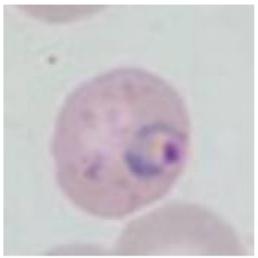


P. falciparum with Maurer's clefts (3)

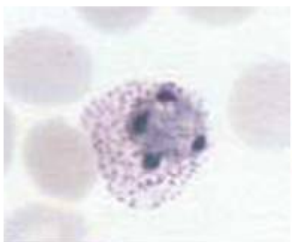
P. falciparum with Maurer's clefts (6)



P. falciparum with basophilic stippling (3)

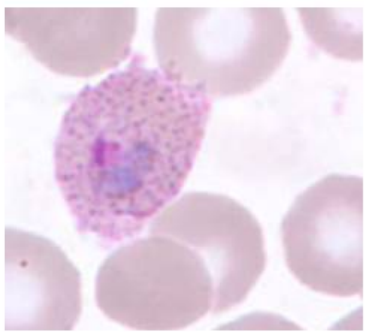


P. malariae with Ziemann's dots. Not as numerous as James' or Schüffner's dots. **NOT SEEN** except with deliberate overstaining.(12)

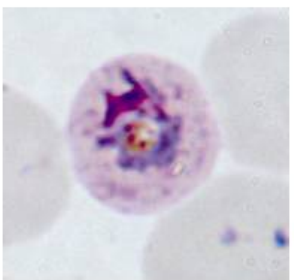


P. vivax ameboid ring with Schüffner's dots (17)

P. vivax with Schüffner's dots (6)



P. ovale trophozoite with James' dots Many authors call these dots "Schüffner's Dots", like the ones in *P. vivax*.(7)



P. knowlesi shows Sinton and Mulligan's stippling under certain staining conditions. (14)

Four Reasons Why Slides From Blood Collected in EDTA Must be Made Within 1 Hour of Blood Draw

1. *P. falciparum* can undergo exflagellation. The flagella may Resemble *Borrelia* spirochetes.

2. *P. falciparum* gametocytes may round up and resemble *P. malariae* gametocytes.

3. *P. vivax* trophozoites may undergo deformation and resemble *P. Ovale* trophozoites

4. In addition, *P. vivax* schizonts can rupture and release merozoites, which can invade new cells, where they may take on the flattened shape of *P. falciparum* "appliqué" forms.

If blood is allowed to sit too long, changes in parasite morphology can result in mistaken identification. Source: Swierczynski & Gobbo, *Atlas of Human Malaria*, 2007, p. 14

Artifacts from *Atlas of Human Malaria*, Swierczynski & Gobbo, 2007

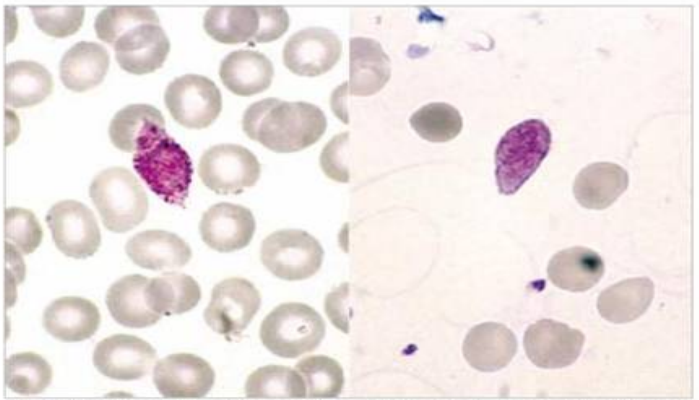


Fig. 433: Right: it's difficult to make a correct diagnosis of this enlarged, oval shaped platelet. Compare with a gametocyte of *P. ovale* (left) where malarial pigment is concentrated towards the periphery of the organism. G. St.

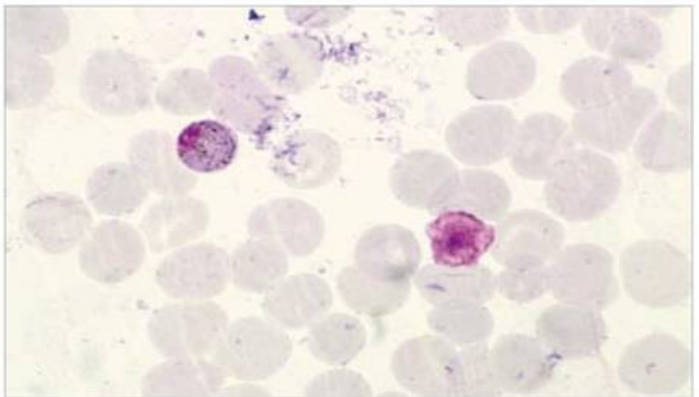


Fig. 434: Left: a *P. malariae* female gametocyte with eccentric nucleus and diffuse pigment; right: an enlarged, round shaped platelet. G. St.

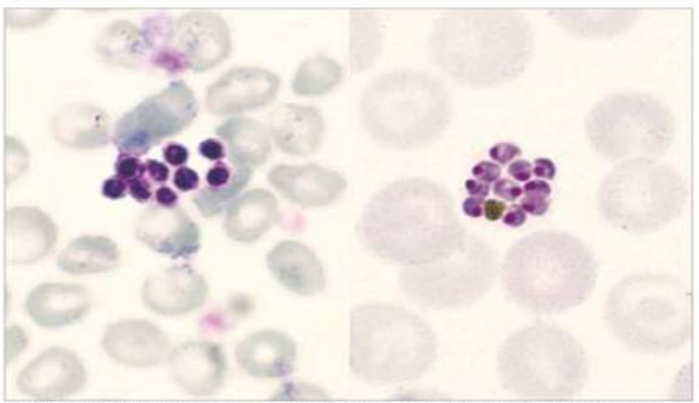


Fig. 435: Left: a cluster of spores may be misidentified as a mature schizont of *P. malariae*. Compare with a mature schizont of *P. malariae* (right): each merozoite displays visible nucleus and cytoplasm. G. St.

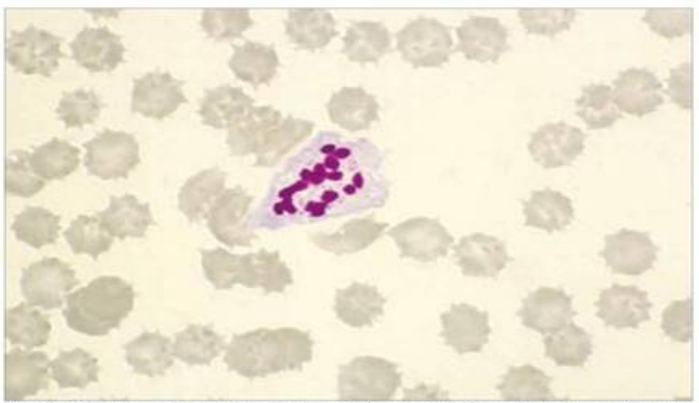


Fig. 436: Pyknotic nuclei in a leukocyte (karyorrhexis and karyolysis) probably due to a prolonged contact with EDTA; these forms should not be confused with merozoites of a mature schizont. G. St.

Geographical Distribution of Human Plasmodia

	P. falciparum	*P. malariae*	*P. ovale*	*P. vivax*
Central Africa	Predominant	rare	rare	rare
East Africa	Predominant	rare	rare	Common
North Africa**	very rare	very rare	Absent	Predominant
West Africa	Predominant	Common	rare	very rare
Central America	Common	rare	Absent	Predominant
South America	Common	Common	Absent	Predominant
Central and southwest Asia	Common	Common	Absent	Predominant
Southeast Europe	very rare	very rare	Absent	Predominant
Indian subcontinent	Common	rare	Very rare*	Predominant
Indochina	Predominant	rare	rare	Common
Indonesia	Predominant	very rare	Very rare	Common
Madagascar, Indian Islands	Predominant	rare	rare	Common
Pacific Islands	Predominant	very rare	rare	Common

The table above is from the "Atlas of Human Malaria" by G. Swierczynski and M. Gobbo, Az Color s.r.l., Sirmione, Italy, 2007, p. 6.

* The Royal Perth Hospital Web site says that some cases of P. ovale are now being identified in the southern states of India

Human cases of *P. knowlesi* infection have been reported in Vietnam, Thailand, Myanmar, peninsular Malaysia, Malaysian Borneo, Singapore, and Palawan Island in the Philippines.

Picture credits:

1. Bannister, L. and Mitchell, G.: The ins, outs and roundabouts of malaria. TRENDS Parasitol 19:209-213 (2003).
2. Royal Perth Hospital. http://www.rph.wa.gov.au/malaria.html
3. Swierczynski, G. and Gobbo, M.: *Atlas of Human Malaria*. Az Color s.r.l., Sirmione, Italy. 2007.
4. GideonOnline. www.GIDEONonline.com. Gideon Informatics, Inc., Los Angeles, CA.
5. Malaria Diagnostics. Presentation by Gail Stennies, Malaria Epidemiology Branch DPD/NCID/CDC May, 2002.
6. *Bench Aids for the Diagnosis of Human Malaria.* 1st ed. World Health Organization, Geneva, 1983.
7. CDC DPDx http://www.dpd.cdc.gov/dpdx/html/malaria.htm
8. U.S. Army Medical Department Center & School. Medical Parasitology CD-ROM. Leishmania-Malaria Course.
9. Field, J.W. *et al*.: *The Microscopical Diagnosis of Human Malaria*. Economy Printers, Kuala Lumpur. 1963.
10. Photograph by Jack Komisar
11. Garcia, L.S.: Malaria. Clinics in Laboratory Medicine. 30:93-129 (2010).
12. *The Microscopic Diagnosis of Malaria.* CD-ROM included with *Basic Malaria Microscopy. Part I. Learner's Guide.* 2nd ed. World Health Organization, Geneva, and Centers for Disease Control and Prevention, Atlanta. 2010.
13. Lontie, M.: *Plasmodia as Seen With the Microscope*. Medisch Centrum Huisarten, Leuven, Belgium. 2001.
14. Lee, K.-S. *et al*.: Morphological features and differential counts of *Plasmodium knowlesi* parasites in naturally acquired human infections. Malaria J. 2009 8:73.
15. Sutamihardja, A. *et al.* (eds.): *Microscopy Malaria Diagnosis*. Department of Medical Parasitology, U.S. Navy Medical Research Unit 2, Jakarta, Indonesia, 2003.
16. Peters, W., and Pasvol, G. *Atlas of Tropical Medicine and Parasitology*. 6th ed. Elsevier Mosby, Edinburgh, 2007.
17. *Bench Aids for the Diagnosis of malaria infections*. World Health Organization, Geneva, 2000.

Binax Instructions

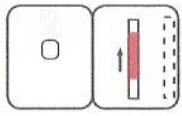
IMPORTANT: The instructions below are abbreviated and are intended for users familiar with the test procedure. Consult the Product Insert for detailed instructions and performance characteristics.

TEST PROCEDURE

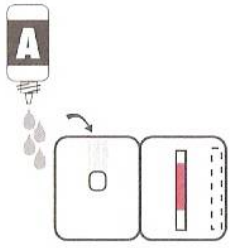
1) **SLOWLY** add 15 µl of blood from an EDTA tube (or from an EDTA capillary tube) to the **PURPLE** sample pad. See figure 1 on test device. **IMPORTANT:** Incorrect addition of sample may lead to an invalid or uninterpretable test.

2) Hold the Reagent A bottle vertically and add **two (2)** free-falling **drops** of Reagent A to the white pad immediately below the purple sample pad. Allow the first drop to absorb into the pad before adding the second drop. See figure 2 on test device.

3) Allow the blood sample to run up the full length of the test strip. See figure 3 on test device. NOTE: If blood flow up the test strip appears to stall or is less than halfway up the strip after one minute, add one additional drop of Reagent A to the white pad at the bottom of the test strip.



4) Just before the blood sample reaches the base of the white pad at the top of the test strip, **SLOWLY** add **four (4)** free-falling drops of Reagent A to the wash pad on the top left-hand side of the device. See figure 4 on test device.

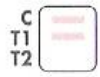


5) Remove the adhesive liner and close the device. See figure 5 on test device.

6) Read the test result 15 minutes after closing the test device.

BinaxNOW® Malaria

RESULT INTERPRETATION

TEST	RESULTS	DESCRIPTION / INTERPRETATION
T1 Positive		Positive result for *P. falciparum* (P.f.)
T2 Positive		Positive result for *P. vivax* (P.v.) or *P. malariae* (P.m.) or *P. ovale* (P.o.) In some cases the appearance of only the T2 Line may indicate a mixed infection with two or more of P.v., P.m., and P.o.
T1 + T2 Positive		Positive result for *P. falciparum* (P.f.) In some cases the appearance of both the T1 and T2 Lines may indicate a mixed infection of P.f. with another species.
No T1 or T2 Lines		Negative result (no malaria antigens were detected)

Invalid and / or Uninterpretable Test Results

The test is invalid if the Control (C) Line does not appear, whether a Test Line(s) is present or not.

The test is uninterpretable if the background color hinders reading of the test result at 15 minutes.

Binax, Inc.
Scarborough, Maine 04074
US: 1-800-637-3717
Outside US: 1-303-530-3888
www.invernessmedicalpd.com

inverness medical
professional diagnostics

Rapid Diagnostic Tests for Malaria

The need for rapid diagnostic tests

The spread of resistance to inexpensive antimalarial drugs, such as chloroquine and sulfadoxine/pyrimethamine among malaria parasites has led to a requirement for expensive drugs to treat malaria, such as artemisinin combination therapy. In order to avoid giving expensive antimalarial drugs to people who do not need them, there is an increased need for specific, reliable, and cost-effective diagnostic techniques. Another reason why specific diagnostic techniques are increasingly important is that reductions in malaria transmission mean that a greater proportion of febrile patients have other, frequently fatal, diseases, which need to be distinguished from malaria (MalERA, 2011). Microscopy is considered the "gold standard" for malaria diagnosis, and the Food and Drug Administration requires data from microscopy to support the licensing of new malaria vaccines and drugs (Wongsrichanalai *et al*., 2007). However, microscopy requires expensive equipment and careful training, and there is a wide variety of levels of competence of microscopists in different settings. Therefore, the development of rapid diagnostic tests, which do not require such training and equipment, and can be done without access to laboratory facilities, has made testing for malaria more widely available, and in some cases more reliable as well. The latest edition (2010) of the WHO Guidelines for the Treatment of Malaria says "Prompt parasitological confirmation by microscopy or alternatively by RDTs is recommended in all patients suspected of malaria before treatment is started. Treatment solely on the basis of clinical suspicion should only be considered when a parasitological diagnosis is not accessible."

This laboratory exercise involves the use of a rapid diagnostic test, BinaxNOW (Alere, 30 South Keller Road, Suite 100, Orlando, FL 32810-6297). The National Stock Numbers for this kit are: 6550015548536 for the 12-test kit, and 65500115548731 for the 25-test kit. The BinaxNOW kit was the first (13 June, 2007) FDA product of its kind (rapid diagnostic test suitable for use in the field) that was cleared for detection and identification of the parasites that cause malaria (A rapid kit called QBC [Quantitative Buffy Coat] was cleared in 1989, but it requires a fluorescence microscope or a special microscope attachment and a centrifuge). As of the time of this writing, January 2013, it is still the only product of its kind that has been cleared by the FDA and is available for sale in the United States. BinaxNOW is an immunochromatographic membrane assay (also known as a "dipstick") that uses monoclonal antibodies to detect a *Plasmodium falciparum*-specific antigen and a

panmalaria antigen that is shared by all species of *Plasmodium*. During clinical trials, BinaxNOW was tested for its ability to detect *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. Sufficient data were collected to support quantitative performance claims for *P. falciparum* and *P. vivax*, but not for *P. ovale* or *P. malariae*. *P. knowlesi* was not tested in the initial trials, but a recent paper reports that heavy infections with *P. knowlesi* can be detected with this kit (Garcia 2010).

The BinaxNOW test kit

What follows is a very abbreviated description of the BinaxNOW test kit. It is essential to read the full-length (about 5 pages) package insert before using the test kit. The abbreviated instructions that are given on the 3 ½" by 6 ¾" card that comes with the test kit, which is reproduced in the laboratory manual, are only intended to be used as a memory aid. The BinaxNOW kit detects the presence of *P. falciparum* by incorporating an antibody to histidine-rich protein 2 (HRP 2), a protein that only *P. falciparum* makes, and an antibody to panmalaria antigen, the enzyme aldolase, to detect *P. ovale, P. malariae*, *P. vivax*, and *P. knowlesi*. The sensitivity of the test varies depending on the species of *Plasmodium* that infects the patient, and a false negative reaction can occur. The package insert gives the expected performance of the test against different concentrations of *P. falciparum* and *P. vivax*. According to the package insert, the sensitivity of the test for *P. falciparum* is more than ten-fold better than the sensitivity for *P. vivax.* The insert also mentions positive test result against *P. malariae* and *P. ovale*, but gives no detailed performance characteristics. The WHO says that the BinaxNOW test is not satisfactory for detecting *P. vivax* in low and moderate transmission areas (WHO Information Note on Recommended Selection Criteria for Procurement of Malaria Rapid Diagnostic Tests 2010.

(www.theglobalfund.org/documents/psm/psm_RDTSelection_Criteria_en/) Tests that are rated by the WHO as satisfactory for the detection of *P. vivax* are described below. Unfortunately, none of these tests are sold in the United States. The sensitivities of rapid diagnostic tests employing the panmalaria antigens pLDH and aldolase for *P. ovale* and *P. malariae* infections are reported to be relatively low (reviewed by Khaimar *et al.,* 2009).

The BinaxNOW test is interpreted as follows: A red line at the HRP2 position (marked "T1") is a positive test for *P. falciparum*. A red line at the panmalaria antigen position (T2) can indicate the presence of *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, *P. knowlesi*, or a mixture of species. If only the panmalaria antigen line is red, then the infection is

probably due to something other than *P. falciparum* (however, antigenic variants of *P. falciparum* HRP2 or deletions of the *hrp-2* genes may not be recognized by monoclonal antibodies). If both lines are red, then the infection may be *P. falciparum* alone or *P. falciparum* plus another species. In all cases, the control line, "C", must also be positive for a test to be valid. A negative BinaxNOW result for a symptomatic patient must be confirmed by a more sensitive test, such as microscopy, and line at the T2 position needs to be followed up by a different kind of test to identify the species (one or more) that is/are present.

The BinaxNOW kit and its reagents are stable from 2°C-37°C (36°F-98.6°F). It is important to use positive controls to test each new shipment or lot to verify that the test is still usable. A clinical laboratory may have its own positive blood samples to use as controls, but if such controls are not available, a positive control kit, catalogue number 665-010, can be purchased from Alere. The National Stock Number for the control kit is 6550015999065.

Transportation and storage of RDTs

The USAID has published and posted online a pamphlet that describes recommended transportation and storage practices for RDTs ("Transporting, Storing, and Handling Malaria Rapid Diagnostic Tests at Central and Peripheral Storage Facilities" http://www.wpro.who.int/NR/rdonlyres/1BBCEFC2-46B3-40F5-9898-7455578145E8/0/MalariaRDT_CENTRAL_2009web.pdf)

It is a challenge to store RDT kits properly in tropical regions, particularly in areas without electricity. For this reason, a low-technology evaporative cooling box for storage of malaria rapid diagnostic tests and other medical devices in areas without electricity has been described by Chanthap *et al.*(2010), and modifications for construction and use in Afghanistan were described by Mikhail *et al*. (2011).

Other rapid diagnostic test kits pair an antibody specific for *P. falciparum* HRP2 with an antibody that recognizes pLDH, parasite lactate dehydrogenase, another protein that is present in all species of *Plasmodium*. Devices that detect LDH require storage at <30°C (ref cited by Drakeley and Reyburn, 2009). Kits based on pLDH (see below) may be more sensitive than the BinaxNOW kit in detecting *P. vivax* (Mikhail *et al*., 2011) and *P. knowlesi* (Garcia, 2010).

Recently, several tests have been brought to market that make use of an antibody to species-specific epitopes of pLDH to detect *P. vivax*. Two of these tests, as well as one test that uses an antibody that reacts with the LDH of all species of *Plasmodium*, are reviewed in Mikhail *et al*. (2011). The tests with the antibody specific for *P. vivax* pLDH are the

CareStart 3-line Pf (HRP2) + Pv (pv-LDH) (Cat No. G0161, Access Bio, Monmouth Junction, New Jersey) and the Bioline 3-line Pf(HRP2) + Pv (pv-LDH) (Cat No. 05FK80, Standard Diagnostics, Gyeonggi-do, Korea). Both tests are described as satisfactory for detection of *P. falciparum* and *P. vivax* in the WHO Information Note cited above, and both tests gave good performance in the studies of Mikhail and coworkers, but the Bioline test gave an unacceptably high proportion of invalid test results (10%) . An invalid result is not a false negative or a false positive, but a test that cannot be read at all. Invalid tests result in a waste of time and money. At the time of writing, these tests could not be purchased in the United States, but they can be purchased in some malaria-endemic countries.

WHO Evaluations of RDTs

More than 150 studies of malaria rapid diagnostic tests have been published. Murray *et al*. (2008) have found methodological flaws in many of these studies. To provide health care workers with reliable information about RDTs, the WHO, beginning in 2008, began to publish a continuing series of reports on the performance of rapid diagnostic tests for malaria (see WHO in the references). Makler and Piper (2009) say the World Health Organization program to evaluate RDTs falls far short of what is needed to understand test performance. However, the WHO reports are the best available information for end users on the reliability of malaria rapid diagnostic tests. The volume of information contained in the online WHO reports is overwhelming and it probably not suitable for laboratories that are just trying to find out which test they ought to obtain. For this purpose, the WHO "Information Note" cited above is especially convenient and easy to use.

RDTs are often superior to microscopy in detecting malaria in pregnancy (reviewed by Unecke 2008). However, HRP2-based RDT's, which are more sensitive than pLDH-based RDTs, fail to detect many placental malaria cases that are detected by placental blood smears (Fried *et al*., 2012). More work is urgently needed to determine the accuracy of RDTs for the diagnosis of placental malaria (Kattenberg *et al*., 2011).

Pros and cons of RDTs

Economic analyses suggest that RDTs could be cost beneficial in all but the highest transmission settings (reference cited by Drakeley and Reyburn, 2009). However, RDTs have several drawbacks, including the limited sensitivity mentioned above. For example, HRP2 is found at detectable levels in some patients 28 days after clinical presentation, well after resolution of symptoms and apparent clearance of parasites from patients (Tjitra *et al*., 2001; reviewed by Murray and Bennett, 2009). This

means that HRP2-based tests are not good for monitoring the response to treatment. In contrast, pLDH is rapidly cleared from the blood following parasite death (World Health Organization 2009). Even so, all current antigen assays may revert to positive if gametocytes subsequently appear in the bloodstream (Murray *et al.,* 2008). Consequently, microscopy, rather than RDTs, should be used to monitor treatment efficacy (Gillet *et al.,* 2010, There are several problems with antigen-based RDTs. For example, HRP2-based tests are subject to the prozone effect, in which very high antigen concentrations cause faint or weaker readings than do lower antigen concentrations (Gillet *et al*. 2009). In addition, false negatives in the HRP2 line have been seen due to mutations in or deletion of the HRP2 and HRP3 genes (see below). The T2 line, containing antibodies to aldolase,will still be positive, but it is less sensitive to *P. falciparum* by an order of magnitude compared to the T1 line. are strains of *P. falciparum* that have mutated or deleted their genes for HRP2 and/or HRP3 (some antibodies to HRP2 also react with HRP3). Those strains will give a negative T1 line, but the T2 line should still be positive if the parasitemia is high enough. But the sensitivity of the test will be lower. As of this writing, reports of negative HRP-2 based tests for P. falciparum have come from the following countries:

Peru, 41% negative (Gamboa *et al*. 2010); 25.7% negative (Maltha *et al*., 2012)

Senegal, 5.9% (7 of 136 samples, Wurtz *et al*. 2013)

Mali, 5.4% (26 of 480 samples, Koita *et al*. 2012)

India, 4.2% (2 of 48 samples, Kumar *et al*. 2013).

Brazil, one case, Houzé *et al*., 2011)

The Centers for Disease Control and Prevention (CDC), with funding from the US Agency for International Development and in collaboration with the Amazon Malaria Initiative, the Pan-American Health Organization, and the Foundation for Innovative New Diagnostics is testing samples from South America for deletion of the HRP-2 and HRP-3 genes. Their July 2012 progress report gave the following results for HRP-2 deletions:

Bolivia, 4% (1 in 25 samples)

Colombia, 7.5% (3 in 40 samples)

Guyana, 0% (0 of 97 samples)

Honduras, 0% (0 of 68 samples)

Peru, 33.3% (31 of 93 samples)

Suriname, 14.10% (11 of 78 samples

Tests based on pLDH appear not to be affected by the prozone effect (Gillet *et al*. 2009). Some RDTs can give false-positive results due to rheumatoid factors, antinuclear antibodies, anti-mouse antibodies, or due to factors that cause sera to be rapid plasma reagin positive (World Health Organization, 2008). RDT's cannot be used to measure parasite density and do not reliably identify the transmission stages of *Plasmodium*, the gametocyte (Drakeley and Reyburn, 2009).

The Use of RDTs in Well-Equipped Laboratories

Although it is necessary to rely on the results of rapid diagnostic tests in situations in which more sensitive tests are not available, they are also useful in well-equipped hospital clinics. The Royal Perth Hospital in Australia describes their policy as follows:

> "We would like to emphasise, that we regard these dipstick methods as useful additional tests to the long established method of examining thick and thin blood films…which is still regarded as the 'gold standard', NOT as replacement methods."
> http://www.rph.wa.gov.au/malaria.html

The utility of rapid diagnostic tests for malaria diagnosis in Africa has been reviewed by Drakeley and Reyburn (2009). The role of diagnostic tests in the eradication of malaria has been reviewed by Tietje *et al.,* 2014.

Products mentioned in this review:

BinaxNOW Malaria Test Kit, manufactured and sold by Alere, 30 South Keller Rd., Suite 100, Orlando, FL 32810-6297, Phone 877-441-7440.

The table below shows the catalogue numbers shown on the package insert from a 25-test BinaxNOW kit and the corresponding National Stock

Number. The 5-test kit, which has been mentioned on some Web sites, was not available at the time of writing.

Product	Cat. No. from insert	National Stock No.
5-test kit	Kit not mentioned	none
12-test kit	665-000	6550015548536
25-test kit	665-025	65500115548731
Positive Control kit	665-010	6550015999065

CareStart 3-line Pf (HRP2) + Pv (pv-LDH), Cat. No. G0161, Access Bio, 2033 US Highway 130 # H, Monmouth Jct, NJ 08852-3003. Phone: (732) 297-2222

Bioline 3-line Pf(HRP2) + Pv (pv-LDH), Cat. No. 05FK80. **Standard Diagnostics** **Address:** 156-68, Hagal-ri, Giheung-eup Yongin-si Gyeonggi-do 449-906, Korea. Phone: 82-31-8999700

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Rapid Tests and Stains

Introduction: There are many requirements and guidelines for collecting different sample types for different body sites that are beyond the scope of this course. However it is important to note that there is ALWAYS the chance of contaminating a swab when collecting a sample. The phrase "junk in, junk out" is often used in the microbiology laboratory: the results can only as good as the sample the lab was given to work with.

SAMPLE COLLECTION

Task 1: Understand the importance of proper specimen collection and handling techniques.

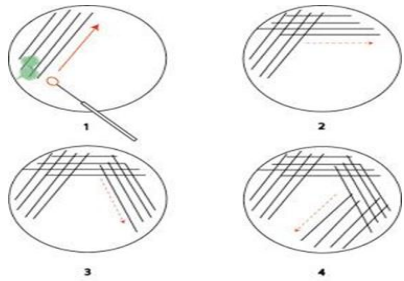
- Use universal precautions for collecting and handling all specimens.
- Whenever possible, collect all culture specimens prior to administration of any antimicrobial agents.
- Avoid contamination with indigenous flora.
- All specimens must be appropriately labeled with two patient identifiers.
- Deliver all specimens to the laboratory as soon as possible after collection. Specimens for bacterial culture should be transported at room temperature. If transport is delayed the following specimens should be refrigerated: urines (within 30 min), stool (within 1 h), respiratory specimens (within 1 h). Specimens for viral culture must be transported to the laboratory immediately on ice. See specific specimen and culture type for detailed collection and transport guidelines.
- Specimens should be in tightly sealed, leak proof containers and transported in sealable, leak-proof plastic bags. Specimens for TB should be double bagged. Specimens

should not be externally contaminated. Specimens grossly contaminated or compromised may be rejected. If anaerobic culture (no air) is requested, make certain to use proper anaerobic collection containers.

WORKING WITH PURE CULTURES

Introduction: Most samples collected will contain several different kinds of bacteria, each with different growth characteristics. Some of these may be contaminants, or normal flora for that body site. Others may be pathogens that are causing an infection, and these should be separated from the contaminants and normal flora for further analysis. Therefore, it is important to isolate a single colony (which contains millions of clones that originally grew from a single bacterium). Microbiologists often use the 4 quadrant streak method to isolate bacteria.

4 Quadrant streak for isolation



Task 2: Perform the 4 Quadrant streak method of isolation

Step 1: Label the bottom of the petri dish with the patient information, type of specimen (i.e. throat, wound, urine, etc.), and date. In a hospital you would also use the unique identifier for that sample, the accession number.

Step 2a: Swab specimen – roll swab on the 1st quadrant, making sure to touch the point of the swab to the media. Be careful not to puncture the media.

Step 2b: Liquid specimen – thoroughly mix the specimen. Use a pipette or sterile inoculating loop to transfer one drop or one loopful to the 1st quadrant area. Spread evenly throughout the 1st quadrant.

Step 3: Rotate the plate. Using a sterile loop, streak the 2nd quadrant. The first 3-5 streaks should completely enter the 1st quadrant, and then subsequent streaks should stay in the 2nd quadrant.

Step 4: Rotate the plate and streak the 3rd quadrant the same way as the 2nd.

Step 5: Rotate the plate and streak the 4th quadrant the same way as the 2nd and 3rd quandrants. Be sure not to reenter the 1st quadrant.

Step 6: Incubate the petri dish under the appropriate conditions (i.e. at 37°C in a CO_2 enriched environment), which depends on the type of bacteria that is suspected.

SAMPLE PREPARATION

Introduction: In order to perform tests to identify the bacteria that grow on the agar plates, a bacterial colony must be examined microscopically. In order to do this, it is transferred from the plate, affixed to a slide, and Gram stained.

Task 3: Prepare a slide for Gram-staining

Step 1a: If the sample is already in liquid form, transfer a drop of the suspended culture to be examined and place it onto a slide with a sterile inoculation loop.

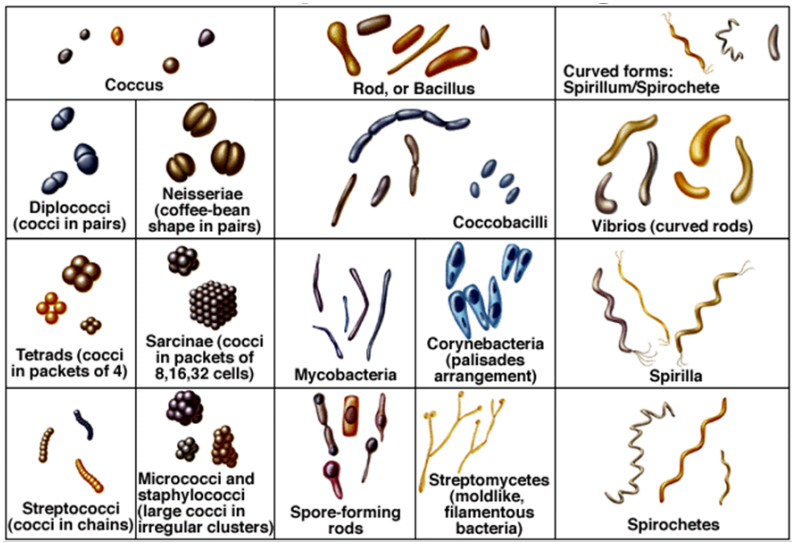
Step 1b: If the sample is to be taken from a petri dish, first add a drop of sterile water on to the slide. Then transfer a tiny amount of a colony from the petri dish, using an inoculating loops or wooden stick (a little goes a long way). Avoid using a cotton swab; the fibers may appear on the stained slide as artifacts. The smear should be thin enough to dry completely within a few seconds.

Step 2: Heat fix the slide either by flame until the slide is too hot to touch or by drying the slide on a slide warmer at 60°C for 10 minutes. Flaming may be bactericidal, so alternately, methanol may be used to fix the sample to the slide. 95% methanol is overlaid on the slide for 1 minute. This method is advantageous because it preserves the morphology of red blood cells as well as bacteria, making it especially useful for examining bloody specimens.

GRAM STAIN

Introduction: The Gram stain is the most commonly used stain in the clinical microbiology laboratory. It is used to divide most bacterial species into two categories: Gram-positive and Gram-negative. The Gram-stain will also tell the microbiologist what the bacteria's shape is, which include cocci (circular), bacilli (rods), diplococci (kidney-beaned shaped cocci in pairs), or coccobacilli (slightly rounded, short rods). Bacteria that do not stain well with the Gram stain or stain variably include organisms such as the spirochetes (i.e. *Treponema* spp., *Leptospira* spp.), *Mycobacteria* spp., and *Gardnerella vaginalis*.

Bacterial Shapes and Arrangements

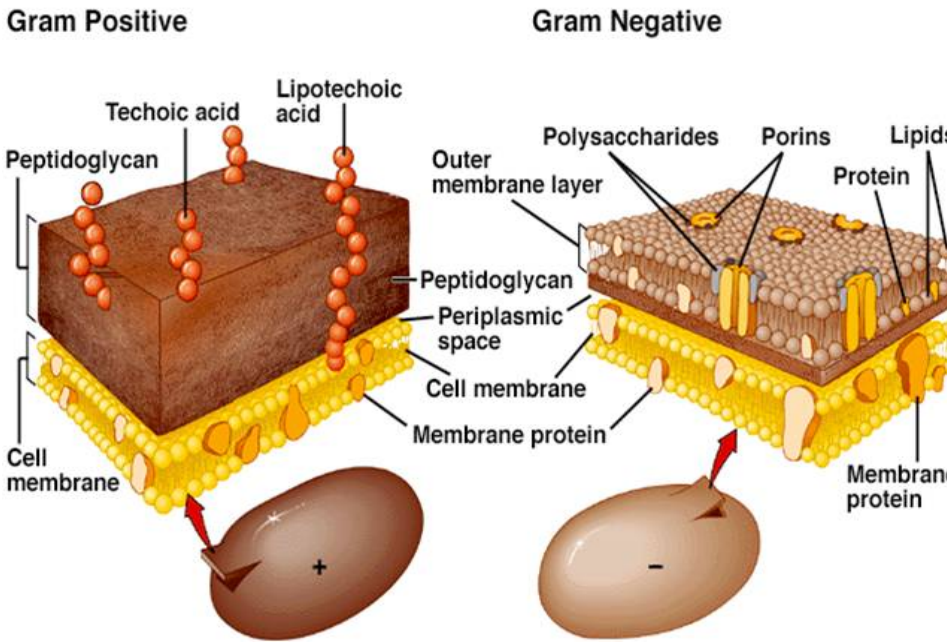


Talaro and Talaro, Foundations in Microbiology, 1999.

Gram-positive bacteria have two layers that make up their cell walls: the inner membrane and a thick layer of peptidoglycan (PG) with numerous teichoic acid cross-linkages. This thick layer of PG readily uptakes the basic primary stain, crystal violet, and the teichoic acids resist decolorization by the acetone alcohol, giving them a blue or purple appearance when examined microscopically. Gram-positive organisms can appear to be Gram-negative with the loss of cell wall integrity, which can occur to antibiotic treatment, old age, or with the action of autolytic enzymes. Yeast typically stain Gram-positive.

Gram-negative bacteria have three layers to their cell walls: the inner membrane, a thin layer of PG, and the outer membrane (made up of lipopolysaccharides and protein islands). The PG layer in Gram-negative bacteria is not sufficient to retain the crystal violet stain, which is readily decolorized by the acetone alcohol. Gram-negative bacteria take up safranin, the counterstain, which gives them a red or pink appearance upon microscopic examination.

Cross sections of Gram-positive and Gram-negative bacterial cell walls:



Talaro and Talaro, Foundations in Microbiology, 1999.

Components of the Gram stain:

1. **Crystal violet** (primary stain): alkaline dye that stains all cells deep blue/purple
2. **Iodine** (mordant): chemically bonds the alkaline dye to the peptidoglycan
3. **95% ethanol/acetone** (decolorizer): washes the crystal violet out of Gram-negative cells, fixes the crystal violet in Gram-positive cells due to dehydration.
4. **Safranin** (counterstain): positively charged dye that will stain Gram-negative cells red/pink. Will also stain eukaryotic cells (i.e. RBCs, epithelial cells) pink.

Gram stains yield results much more quickly than culture, which is especially important when infection would make an important difference in the patient's treatment and prognosis (i.e. bacterial meningitis, bloodstream infections). The types of bacteria that are reported depend on the laboratory, the specimen site, and what is considered normal flora. Slides are first examined on low power to look for large structures such as hyphae and blood cells. Oil immersion allows for viewing bacteria and cell morphology. For specimens where organisms and blood cells are quantified, such as tissue and swabs, the quantity is determined by averaging the number of bacteria in 30-40 fields on the slide, skipping areas where no bacteria are present.

Task 4: Perform the Gram stain procedure

Step 1: Take the fixed slide from Task 2 and place on a wire rack over a laboratory sink.

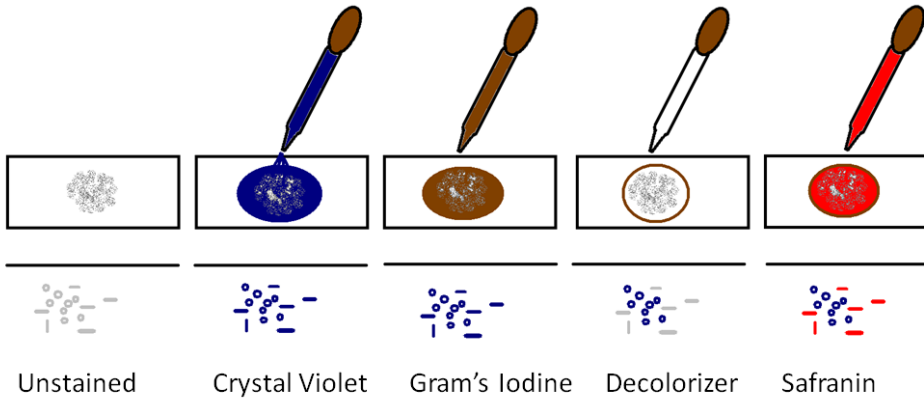
Step 2: Flood the slide with crystal violet for 60 seconds, rinse the slide with tap water

Step 3: Flood the slide with iodine for 60 seconds, rinse the slide with tap water

Step 4: Decolorize the slide by lifting one end and running the ethanol acetone down the slide for about 5 seconds, rinse the slide with tap water

Step 5: Flood the slide with safranin for 30 seconds, rinse with tap water and allow the slide to dry (does not have to be completely dry for microscopic examination)

Step 6: Examine the slide under the microscope using a 100x objective under oil immersion



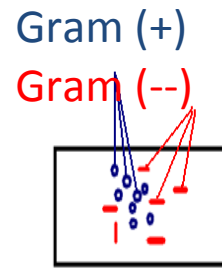


Illustration by Amanda Pierson, WRAIR Staff Graphic Design Artist

Gram-negative bacilli (Escherichia coli)

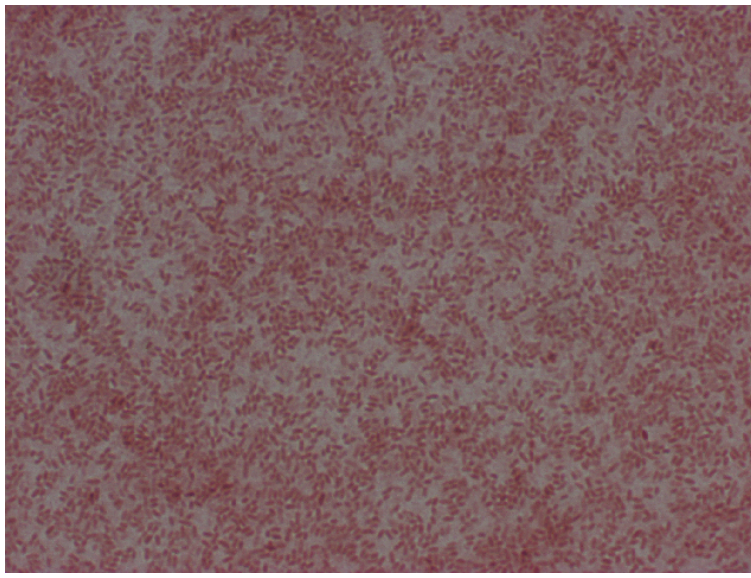
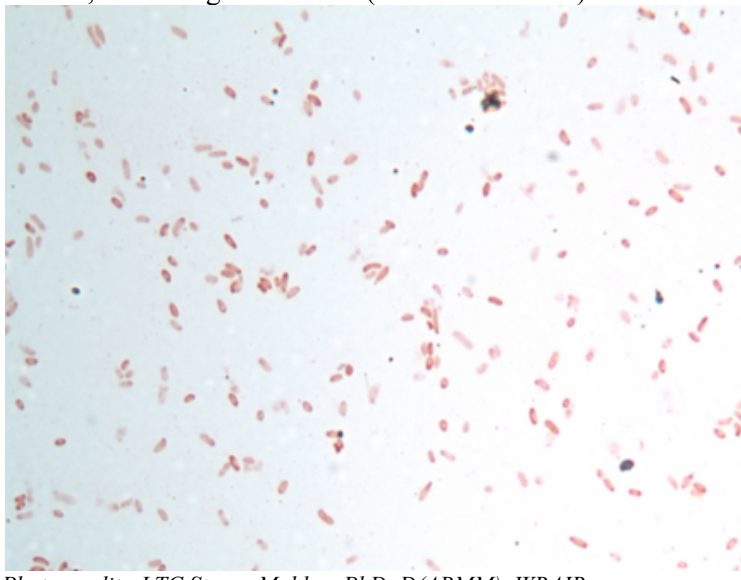


Photo credit: Amy Summers, MT(ASCP), WRAIR

Curved, Gram-negative bacilli (Vibrio vulnificus)



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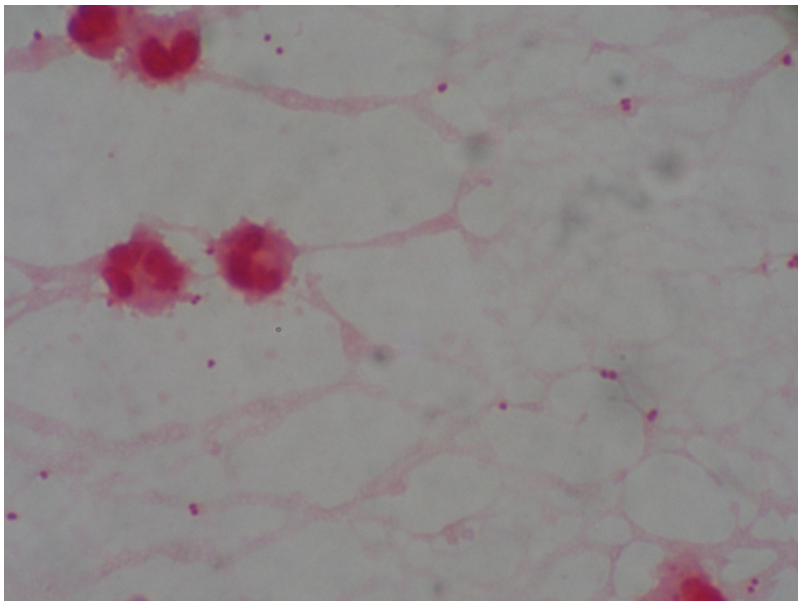
Gram-negative diplococci (Neisseria meningitidis)



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Gram-positive cocci in clusters (Staphylococcus aureus)

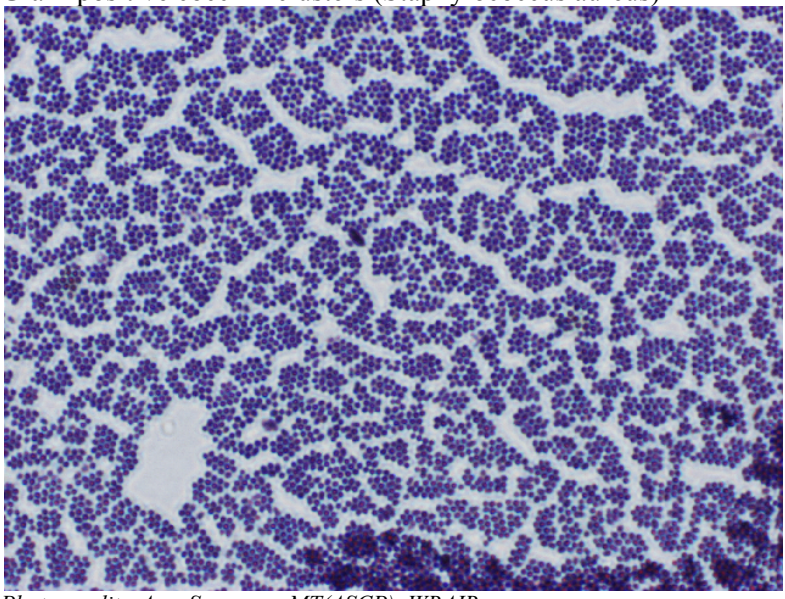
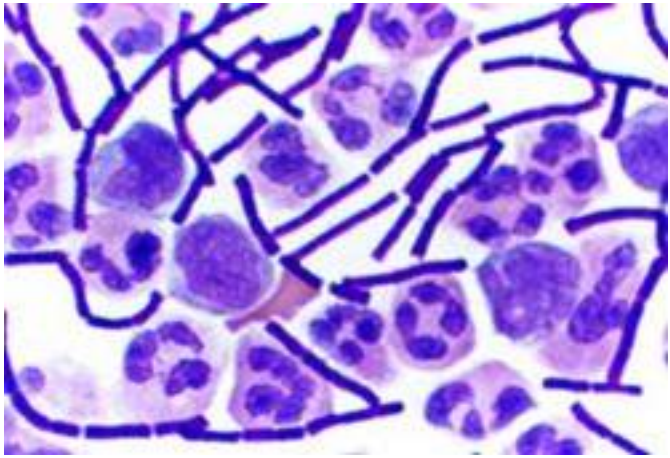


Photo credit: Amy Summers, MT(ASCP), WRAIR



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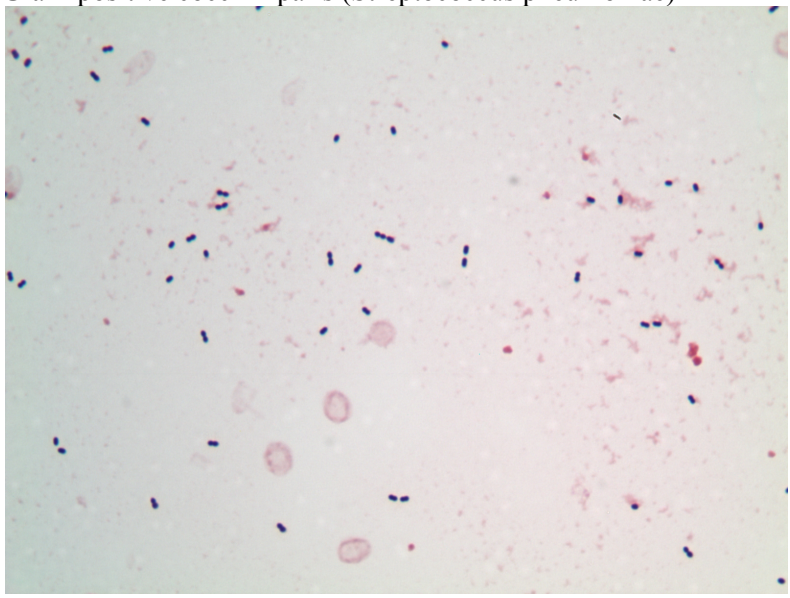
Gram-positive cocci in pairs (Streptococcus pneumoniae)



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Gram-positive bacilli (Clostridium perfringens)

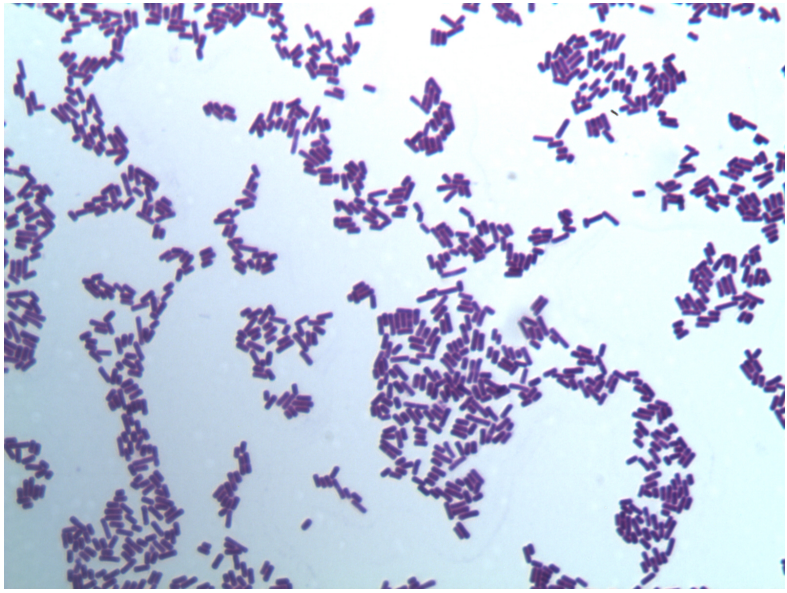


Photo credit: LTC Steven Mahlen, PhD, D(ABMM), WRAIR

Budding yeast (Candida albicans)

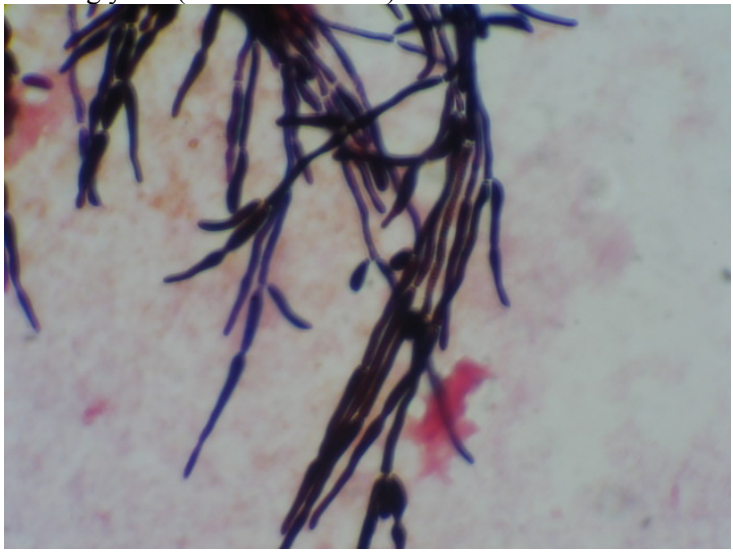


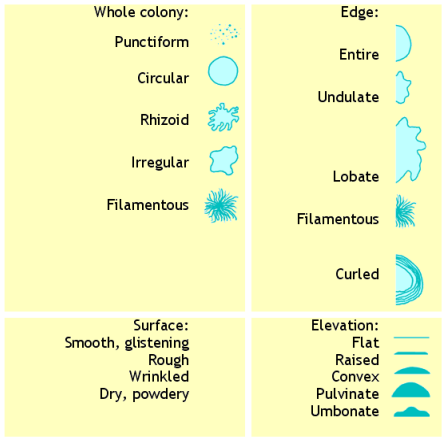
Photo credit: LTC Steven Mahlen, PhD, D(ABMM), WRAIR

GROWTH ON AGAR MEDIA

Introduction: Bacteria that grow on agar plates have many different morphological appearances (colony size, color, hemolysis). They also have different growth requirements and often produce different metabolic waste products. Microbiologists exploit these characteristics and differences to presumptively identify bacterial species.

Different types of agar plates can be used to grow different bacteria. Enriched media like blood and chocolate agar support the growth of nutritionally fastidious organisms and are used routinely for many different sample types and culture sites. Selective media inhibit the growth of certain organisms with dyes, antibiotics or salts while promoting the growth of others (i.e. CNA, mannitol salt, MacConkey agar). Differential media use agents such as sugars with pH indicators to give certain organisms a distinct colony appearance (i.e. mannitol salt, MacConkey agar). It should be noted that different media often combine the advantages of being both selective and differential.

Appearance on blood agar plate



Talaro and Talaro, Foundations in Microbiology, 1999.

Hemolysis on blood agar media

Alpha hemolysis refers to the partial lysis of red blood cells and hemoglobin. This results in a greenish-grey discoloration of the blood around the colonies.

Beta hemolysis is the complete lysis of red blood cells and hemoglobin. This results in complete clearing of the blood around the colonies.

No hemolysis, sometimes called gamma hemolysis results in no change in the medium.

Photo credit: Amy Summers, MT(ASCP), WRAIR

Task 5a: Describe colony morphology of an unknown organism on a blood agar plate.

Step 1: Record the different morphological characteristics of the organism on a blood agar plate, including color, colony shape and size, edge, surface, surface, elevation, and hemolysis.

Task 5b: Use differential growth media to presumptively identify an organism
selective media (i.e. MacConkey, CNA agar).

MacConkey agar is both selective and differential. It contains bile salts and the dye crystal violet, which inhibit the growth of gram-positive bacteria and select for gram-negative bacteria. It also contains the carbohydrate lactose, which allows differentiation of gram-negative bacteria based on their ability to ferment lactose. Organisms which ferment lactose produce acid end-products which react with the pH indicator neutral red, and produce a pink color.

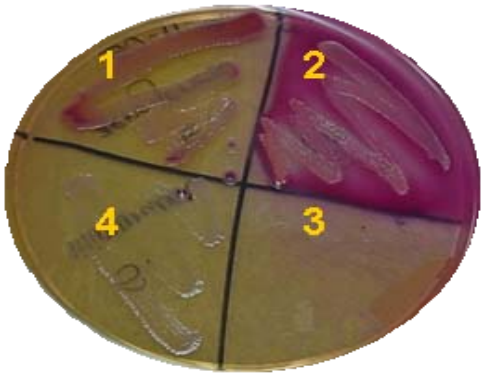


Photo credit: Amy Summers, MT(ASCP), WRAIR

Quadrant 1: The organism is not inhibited by bile salts and crystal violet and is a Gram-negative bacterium. The pink color of the bacterial growth indicates that it is able to ferment lactose.

Quadrant 2: Growth on the plate indicates the organism is not inhibited by bile salts and crystal violet and is a Gram-negative bacterium. The pink color of the bacterial growth indicates it is able to ferment lactose.

Quadrant 3: Absence of growth indicates the organism is inhibited by bile salts and crystal violet and is a Gram-positive bacterium.

Quadrant 4: Growth on the plate indicates the organism is not inhibited by bile salts and crystal violet and is a Gram-negative bacterium. The absence of color in the bacterial growth indicates that the organism is unable to ferment lactose.

Columbia CNA Agar with 5% Sheep Blood: The addition of the antimicrobial agents, colistin and nalidixic acid, renders the medium selective for gram-positive microorganisms. The colistin disrupts the cell membranes of gram-negative organisms, whereas the nalidixic acid blocks DNA replication in susceptible gram-negative bacteria.

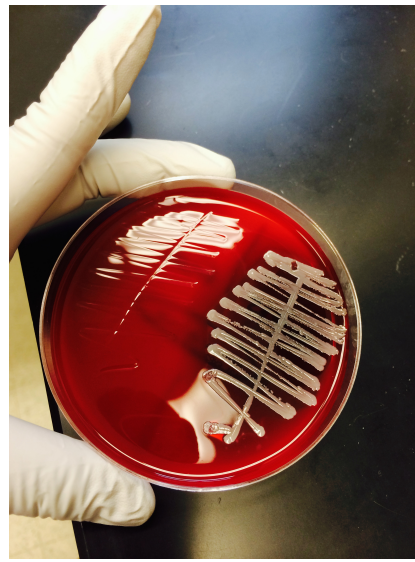


Photo credit: Amy Summers, MT(ASCP), WRAIR

Left Side: Absence of growth on the plate indicates the organism is sensitive to the antibiotics and is Gram-negative.

Quadrant 2: Growth on the plate indicates this organism is resistant to the antibiotics colistin and naladixic acid and is Gram-positive.

RAPID BIOCHEMICAL TESTS

Introduction: After separating bacteria by shape and Gram-stain appearance, microbiologists use additional biochemical tests to further aid in the identification of the organism.

Catalase is an enzyme that breaks hydrogen peroxide down into oxygen and water (positive test is visualized by rapid bubbling). Negative test will not bubble.

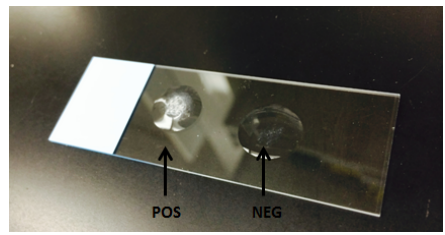


Photo credit: Amy Summers, MT(ASCP), WRAIR

The **coagulase** test is useful in differentiating *Staphylococcus aureus* from other coagulase negative *Staphylococci.* Staphaurex, a rapid coagulase test, consists of white latex particles coated with human fibrinogen for detection of clumping factor and coated with specific IgG for detection of protein A. If the test organism is *Staphylococcus aureus*, it will clump after being added to the reagent and rotated for 20 seconds. If the test organism is negative, the reagent/organism mixture will stay white and diffuse after 20 seconds of rotation.

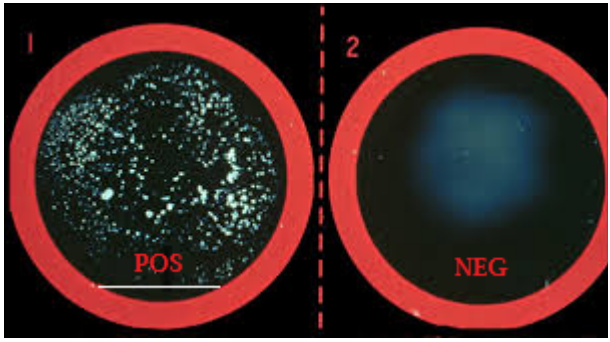


Photo credit: Amy Summers, MT(ASCP), WRAIR

The **oxidase** test identifies organisms that produce the enzyme cytochrome oxidase. The oxidase reagent contains a chromogenic reducing agent, which is a compound that changes color when it becomes oxidized. If the test organism produces cytochrome oxidase, the oxidase reagent will turn blue or purple within 15 seconds.

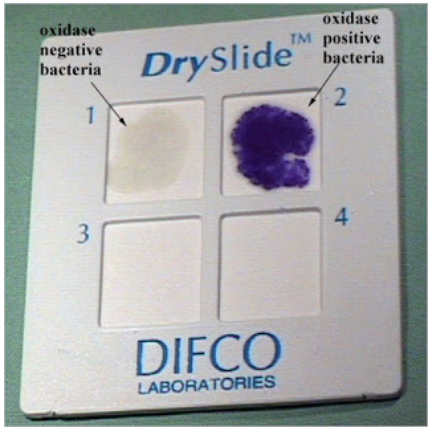


Photo credit: Amy Summers, MT(ASCP), WRAIR

The **indole** test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole (tryptophan + water = indole + pyruvic acid + ammonia). A positive result is the appearance of a pink-red color.

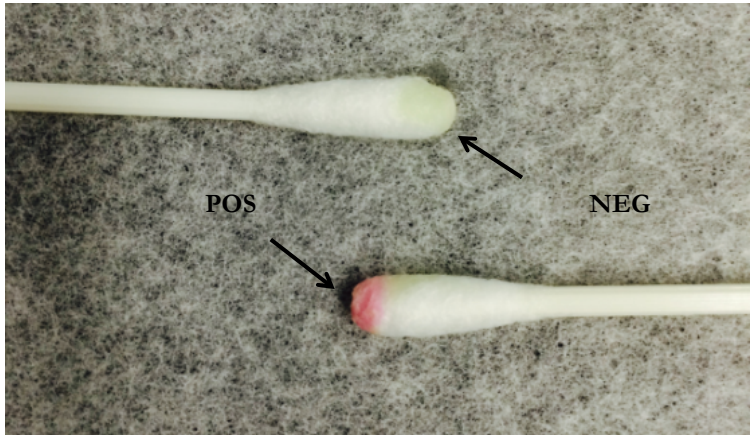


Photo credit: CPT Amanda Roth, WRAIR

The **Enterotube™ II** is a rapid, multi-test system used in identification of unknown Gram-negative bacilli that are oxidase-negative (Enterobacteriaceae family). The flat sided tube contains 12 compartments that each contain a different biochemical test. It is used by first removing the caps from both ends to expose the sterile inoculation wire. The Enterotube™ II is inoculated by touching the wire to an isolated colony from a petri dish. The wire is pulled and rotated from the other end to inoculate all 12 compartments and then pushed back in to reinoculate them. The wire is then pulled until it reaches the indole compartment and is borken off using a pair of pliers. The performation of the aerobic compartments must be punctured using a sterile inoculation needle or similar tool. The tube is recapped and incubated at 37°C for 24 hours. After 24 hours of incubation, any color change is recorded in the Identification Card provided by the manufacturer. Two comparments, indole and Voges-Prosckauer, require the addition of reagents before results can be recorded. The numerical values of the positive tests are added in their appropriate sections to yield a 5-digit ID for the organism being tested. This 5-digit number is compared to a reference book or software program to determine the bacterial identification.

Inoculation of the Enterotube™ II

Photo credit: SPC Bartholomew Taylor, WRAIR

Enterotube™ II Interpretation Guide

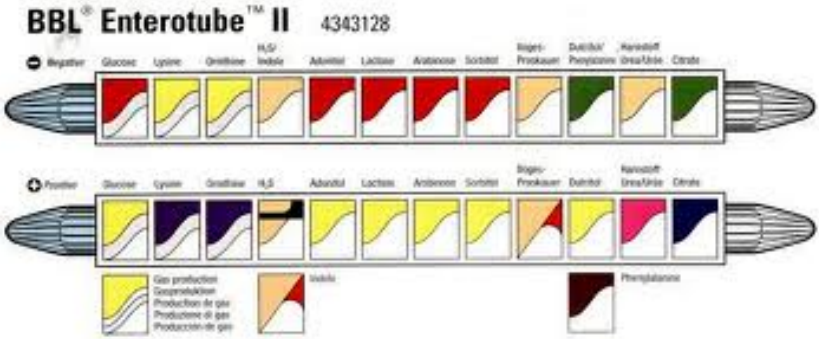


Figure provided by BD BBL

Task 6: Perform rapid biochemical tests that help can provide preliminary identification of bacterial isolates (blood agar plate and colony morphology, catalase, oxidase, indole)

Task 6a: Perform the catalase test on two bacteria and record the results

Step 1: Transfer a visible amount of bacteria to a glass slide

Step 2: Add a drop of 3% hydrogen peroxide to the bacteria, examine immediately to record results.

Task 6b: Perform the rapid coagulase test (Staphaurex) on two bacteria and record the results

Step 1: Transfer 5-6 well isolated colonies to two separate black circles

Step 2: Add one drop of gently, but well mixed reagent to each circle and mix thoroughly with the wooden applicators provided. Be sure to use a different stick for each circle

Step 3: Rotate card for 20 seconds and record results

Task 6c: Perform the oxidase test on two bacteria and record the results.

Step 1. Collect a visible amount of colonies on a wooden applicator and apply to one unused corner of the Oxidase Dryslide media. Wit 15-20 seconds and record results.

Task 6d: Perform the indole test on two bacteria and record the results.

Step 1: Collect a visible amount of colonies on the swab.

Step 2: Apply 1-2 drops of the indole reagent to the swab. Wait 1-3 minutes and record results.

Task 6e: Correctly identify the organism that was inoculated in the Enterotube™II

Step 1: Record the colors in the 12 different compartments of the Enterotube™II and compare them to the interpretation guide.

Step 2: Add the numerical values for each section's positive compartments (should result in a 5-digit number.

Step 3: Look up that 5-digit number for the bacterial identification and record the results.

Preparing a blood smear

Introduction: An acceptable blood smear needs to be made prior to staining and examination (parasites, WBC differential, bacteria, etc.) An acceptable blood smear should have the following characteristics:

- Should cover approximately half of the slide, tapering at the end with a feathered edge
- Should not extend to the edges of the slide
- Should be uniform in consistency (i.e. without holes, scratches, or ridges)

The characteristics of a blood smear are affected by the size of the drop of blood, the angle at which the spreader slide is held and the spped at which the drop is spread. These factors affect the amount of blood carried by the spreader slide which then affects the thickness of the smear. It takes a lot of practice to consistently make a good blood smear.

Task 8: Prepare a blood smear for microscopic examination

Step 1: Lay a clean microscope slide on a flat surface

Step 2: Place a small drop of blood about half an inch from the frosted end of the slide using an applicator stick, capillary tube, or needle.

Step 3: Lightly balance another slide (the spreader slide) on your fingertips and place the spreaders slide at a 30 degree angle in front of the drop of blood

Step 4: Pull the spreader slide back toward the blood droplet until it touches the droplet and blood spreads along the edge of the spreader slide

Step 5: Quickly push the spreader slide forward using a steady, even motion. The weight of the slide is the only pressue applied to the smear during this procedure.

Step 6: Air dry the smear and stain within one hour

The Diff-Quick® Stain

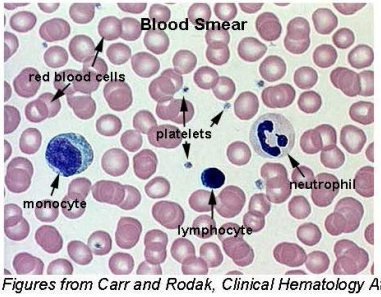
Introduction: Diff-Quick® is a proprietary, Romanowski stain containing a mixture of eosin and methylene blue. When applied to blood cells that are affixed to a slide, the dyes produce multiple colors basic on the ionic charge of the stain and various cellular components. Eosin ions are negatively charged and stain basic cellular components an orange to pink color. Methylene blue ions are positively charged and stain the acidic cellular components in varying shades of blue. Neutral components of the cell are stained by both eosin and methylene blue, producing variable colors.Diff-Quick® staining components.

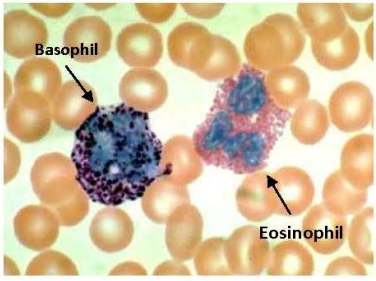
Figure provided by Fisher Scientific

Appearance of different components in a peripheral blood smear:

	Cellular Component	Color	Increase	Associated pathological conditions
Red blood cells	NA	Pink	Polycythemia	Myeloproliferative disease
Platelets	NA	Violet to purple	Thrombocytosis	Myeloproliferative disease, inflammation
Neutrophils	Nucleus	Dark	Neutrophilia	Bacterial infection,

		blue		
	Cytoplasm	Pale pink		acute inflammation, certain cancers
Eosinophils	Nucleus	Blue	Eosinophilia	Allergic reaction, parasitemia, certain cancers
	Cytoplasm	Blue pink to red to orange		
	Granules			
Basophils	Nucleus	Purple or dark blue	Basophilia	Allergic reaction, inflammation
	Granules	Dark purple to black.		
Monocytes	Nucleus	Violet Sky blue	Monocytosis	Chronic-inflammation, certain cancer
	Cytoplasm			
Lymphocytes	Nucleus	Blue to purple Light blue	Lymphocytosis	Acute viral infeciton, Chagas Disease, certain cancers
	Cytoplasm			





Figures from Carr and Rodak, Clinical Hematology Atlas, 2004

Task 9: Perform a Diff-Quick® stain on a freshly prepared blood smear for microscopic examination.

Step 1: Dip the blood smear into the Diff-Quick® Fixative Solution five times for one (1) second each time. Wipe the back of the slide with gauze to prevent carry over into the next stain. Do not allow the fixative to dry on the slide as this will result in an over-fixed wet

appearance. Immediately proceed to the next step while the slide is still wet.

Step 2: Dip the fixed blood smear into the Diff-Quick® Solution I five times for one (1) second each time. Wipe the back of the slide with gauze to prevent carry over into the next stain. Do not allow the stain to dry on the slide. Immediately proceed to the next step while the slide is still wet.

Step 3: Dip the fixed blood smear into the Diff-Quick® Solution II five times for one (1) second each time. Wipe the back of the slide with gauze to prevent carry over into the next stain. Do not allow the stain to dry on the slide. Immediately proceed to the next step while the slide is still wet.

Step 4: Rinse the fixed blood smear with distilled water five times for one (1) second each time. Wipe the back of the slide to decrease drying time. Set the slide upright to air dry. Allow the slide to dry thoroughly before reading.

Step 5: To decrease drying time, slides may be blown dry. Set the blow dryer on cool and hold the slide at least 12 inches from the air flow until completely dry.

Step 6: Look at the morphology of the various cellular components of peripheral blood under a microscope on 50x and 100x objectives with oil immersion.

RAPID ANTIBODY/ANTIGEN TESTS

Introduction: The body's response to infection includes antibody production. The laboratory takes advantage of the production of these antibodies to help identify that exposure to an infectious agent has occurred. Often the lab will request paired samples to compare titers. Common FDA cleared antibody/antigen tests in the microbiology laboratory include (but are not limited to) those for the detection and/or identification of HIV, beta-hemolytic Streptococcal groups, and influenza.

The **OraQuick ®** ***ADVANCE*** rapid test utilizes a proprietary lateral flow immunoassay procedure. The assay test strip contains synthetic

peptides representing the HIV envelope region in the Test (T) zone and a goat anti-human IgG in the Control (C) zone immobilized onto a nitrocellulose membrane. An oral fluid specimen is collected using the flat pad on the test device, followed by insertion into the developer solution. If the specimen contains antibodies that react with the antigens immobilized on the nitrocellulose membrane, a reddish-purple line will appear, qualitatively indicating the presence of antibodies to HIV-1 and/or HIV-2 in the specimen. A built-in procedural control serves to demonstrate that a specimen was added to the vial and that the fluid has migrated adequately through the test device (C zone). A reddish-purple line will appear in the C zone during the performance of all valid tests, whether or not the sample is positive or negative for antibodies to HIV-1 and/or HIV-2.

OraQuick ® ADVANCE Rapid Test Device for the presence of anti-HIV antibodies

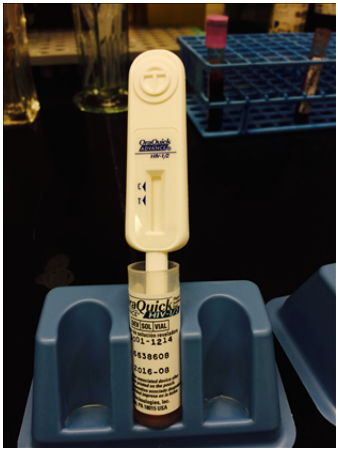


Photo credit: CPT Amanda Roth, WRAIR

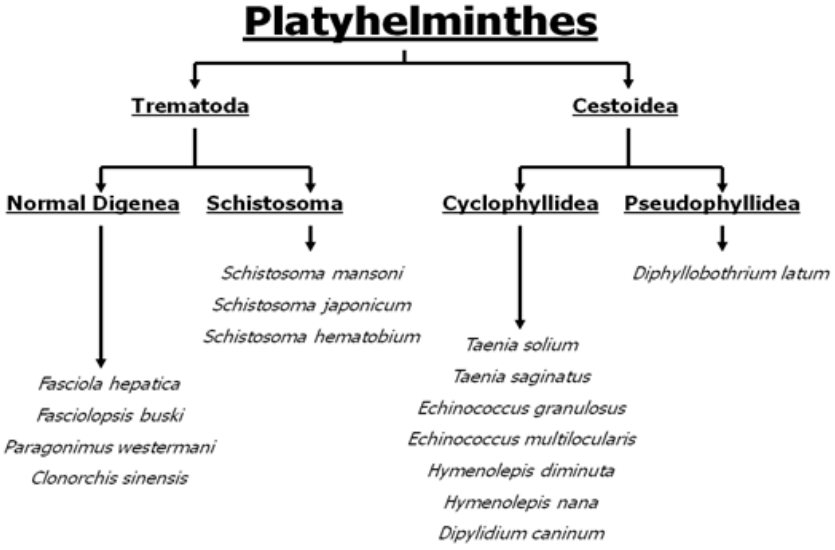
Introduction: Group A *Streptococcus, Streptococcus pyogenes,* is the most significant cause of pharyngitis. Early diagnosis and treatment of Group A streptococcal pharyngitis has been shown to reduce the severity of symptoms and serious complications such as rheumatic fever and glomerulonephritis.

The **Quidel QuickVue Strep A Test** is a lateral flow immunoassay that allows for the rapid detection of Group A Streptococcal antigen directly from patient throat swab specimens. It is a one reagent test that gives results in 5 minutes. The test is intended for use as an aid in the diagnosis of Group A Streptococcal infection. Extracted solution from the throat swab is applied to the test strip and flows through the label pad by capillary action. If the solution contains the Strep A antigen, a red test line will form on the label pad. A blue control line will also form, indicating the test was performed correctly.

Ova and Parasites

In this laboratory exercise, an appreciation of the various types of major parasitic groups will be examined, examples of the major groups will be viewed under a microscope to appreciate the various presentations of the parasites, and discussion will be exchanged about the relevance of major parasitic groups. The three major groups are illustrated below:

The first group to be examined will be the flatworms (or Platyhelminthes). The relationship of various groups within this major division is illustrated below:



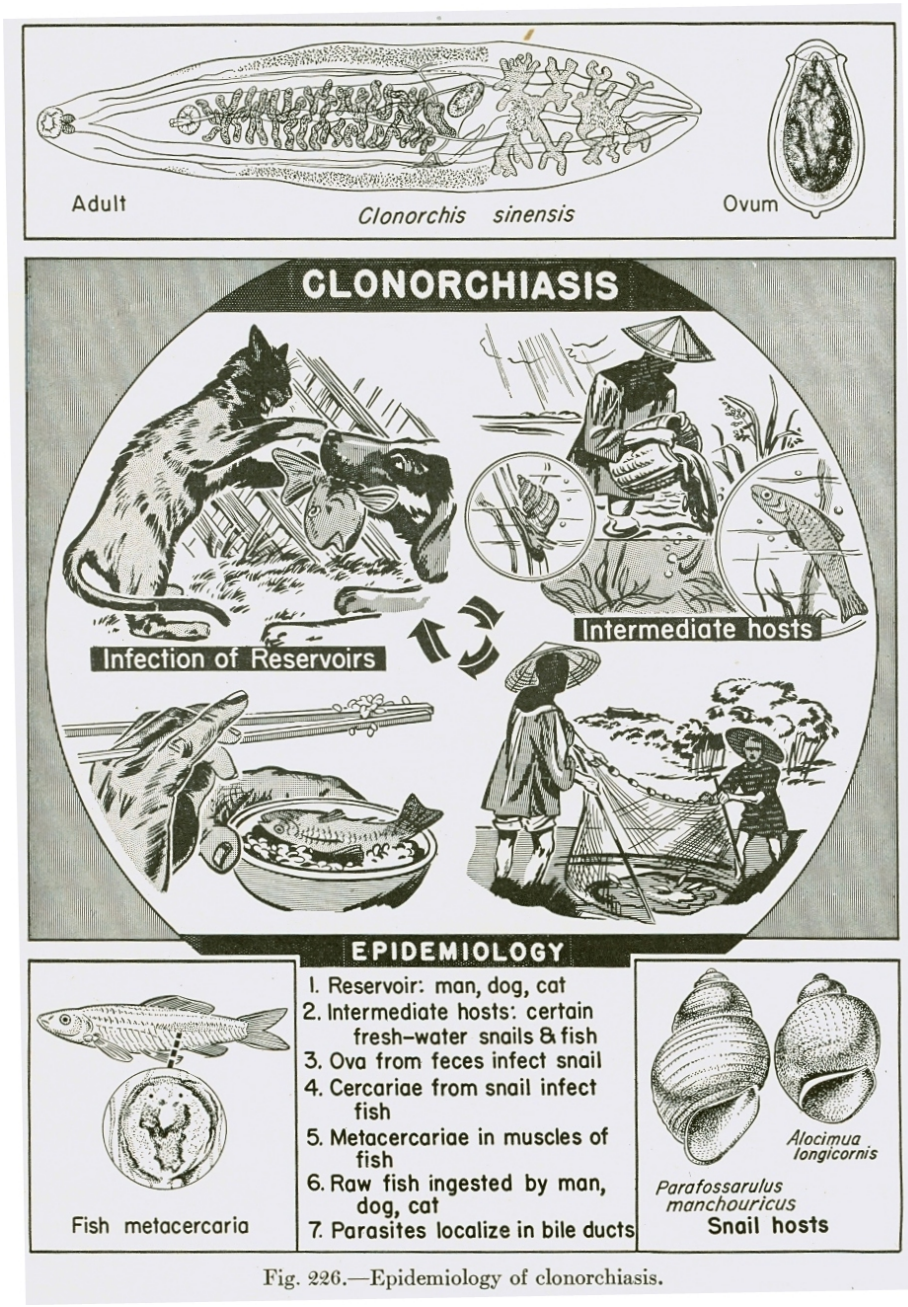


Fig. 226.—Epidemiology of clonorchiasis.

(Courtesy of A Manual of Tropical Medicine by Thomas T. Mackie, George W. Hunter III, and C Brooke Worth, 1945 by W. B. Saunders Company, Philadelphia)

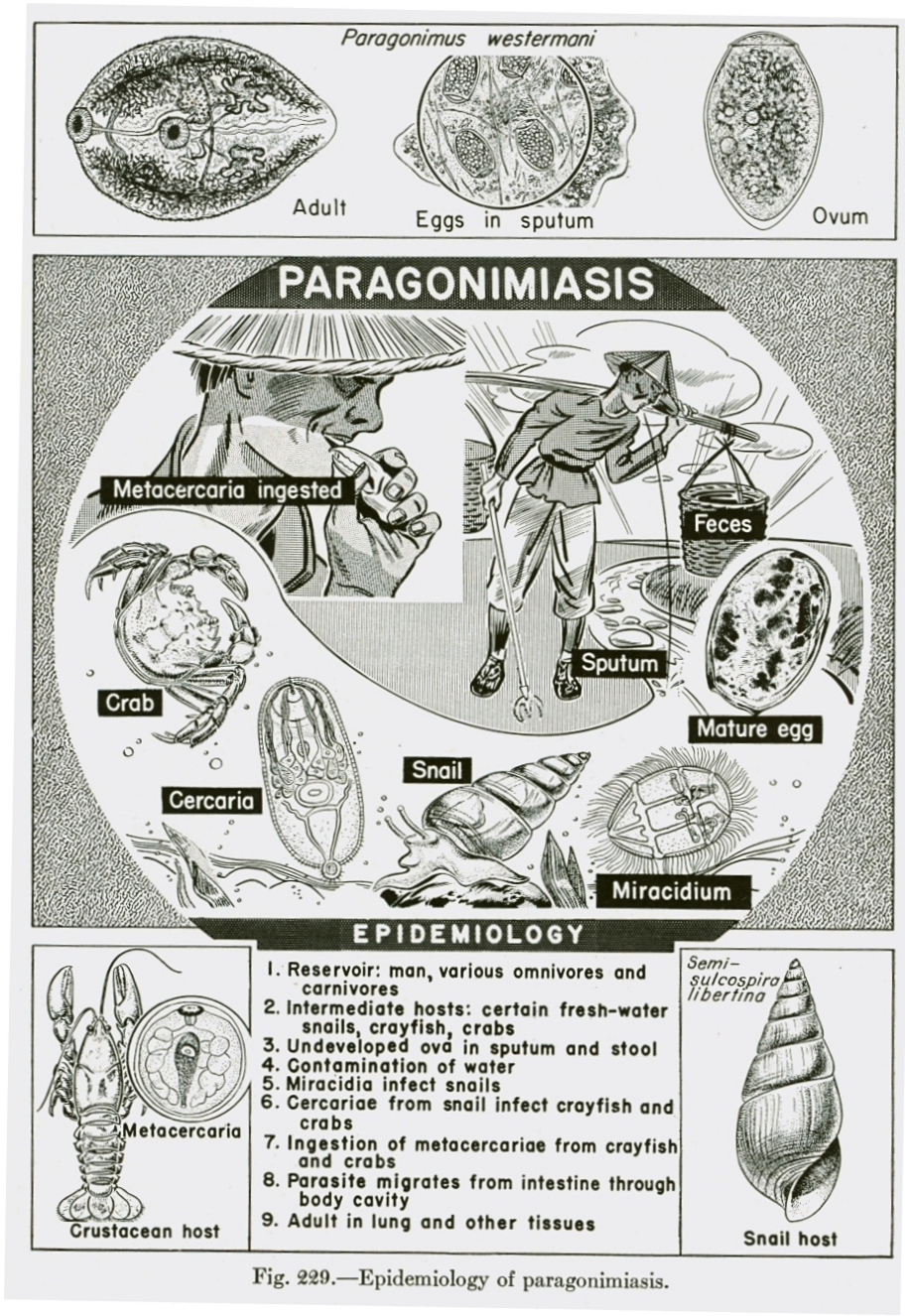


Fig. 229.—Epidemiology of paragonimiasis.

(Courtesy of A Manual of Tropical Medicine by Thomas T. Mackie, George W. Hunter III, and C Brooke Worth, 1945 by W. B. Saunders Company, Philadelphia)

Fig. 213.—Epidemiology of the schistosomiases.

(Courtesy of A Manual of Tropical Medicine by Thomas T. Mackie, George W. Hunter III, and C Brooke Worth, 1945 by W. B. Saunders Company, Philadelphia)

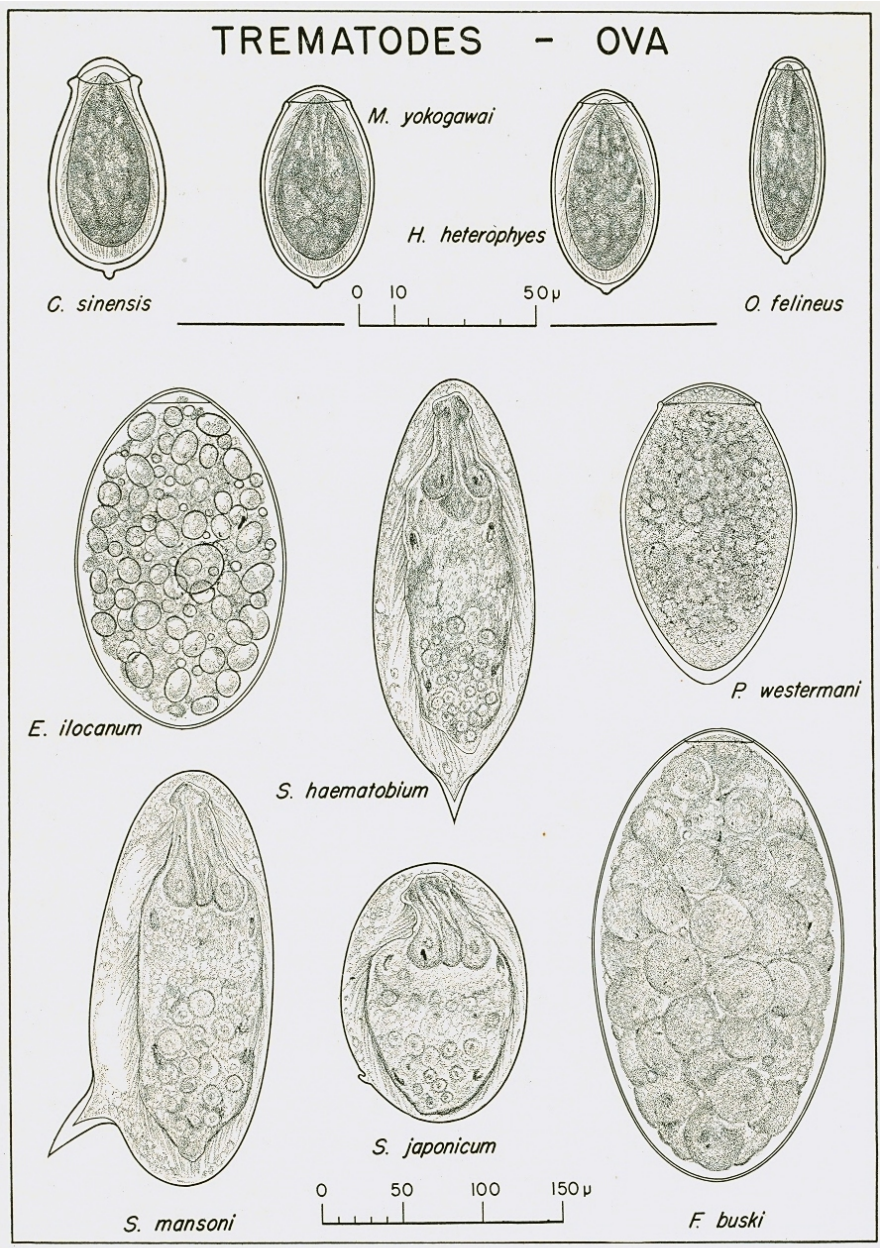
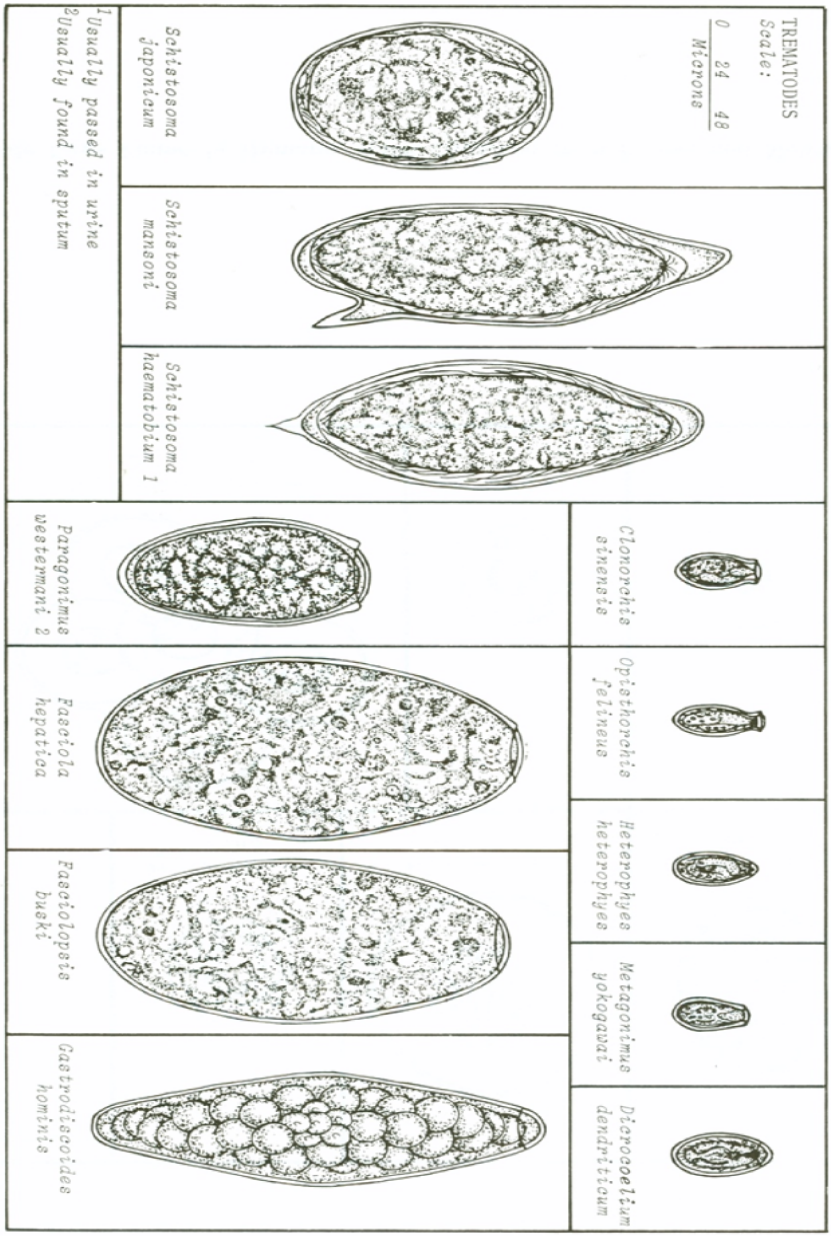


Fig. 222.—Trematode ova.

(Courtesy of A Manual of Tropical Medicine by Thomas T. Mackie, George W. Hunter III, and C Brooke Worth, 1945 by W. B. Saunders Company, Philadelphia)



(Courtesy of the Army / Air Force manual AFM 160-48/TM 8-227-2, 1August 1974)

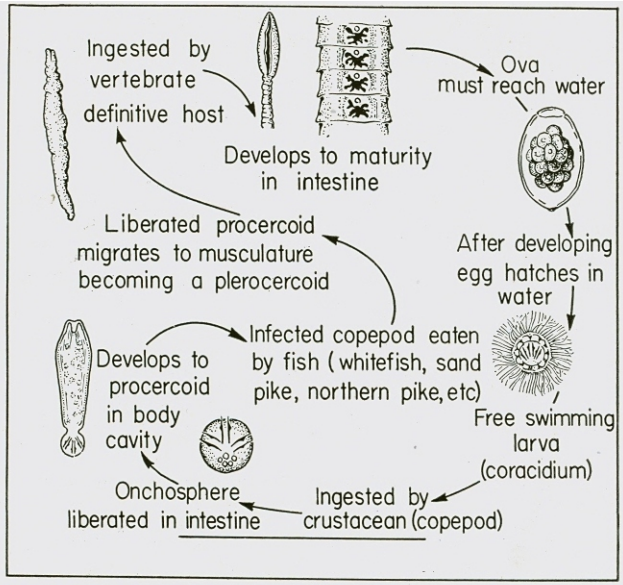


Fig. 233.—Cestode cycle—*D. latum* type.

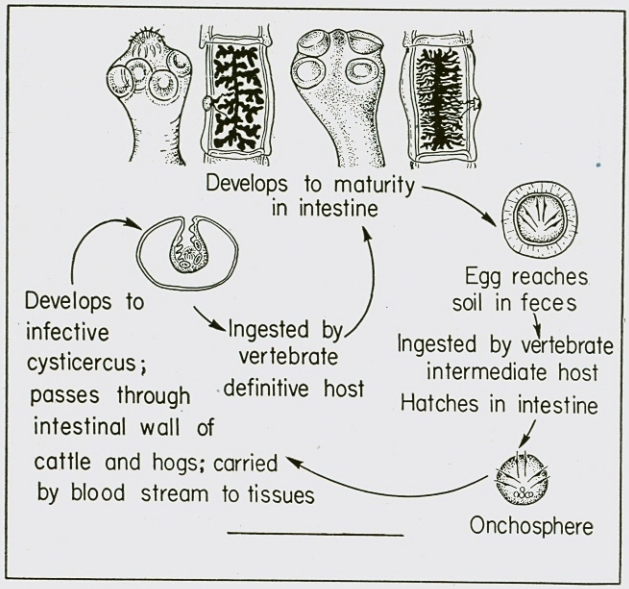


Fig. 234.—Cestode cycle—*Taenia* type.

(Courtesy of A Manual of Tropical Medicine by Thomas T. Mackie, George W. Hunter III, and C Brooke Worth, 1945 by W. B. Saunders Company, Philadelphia)

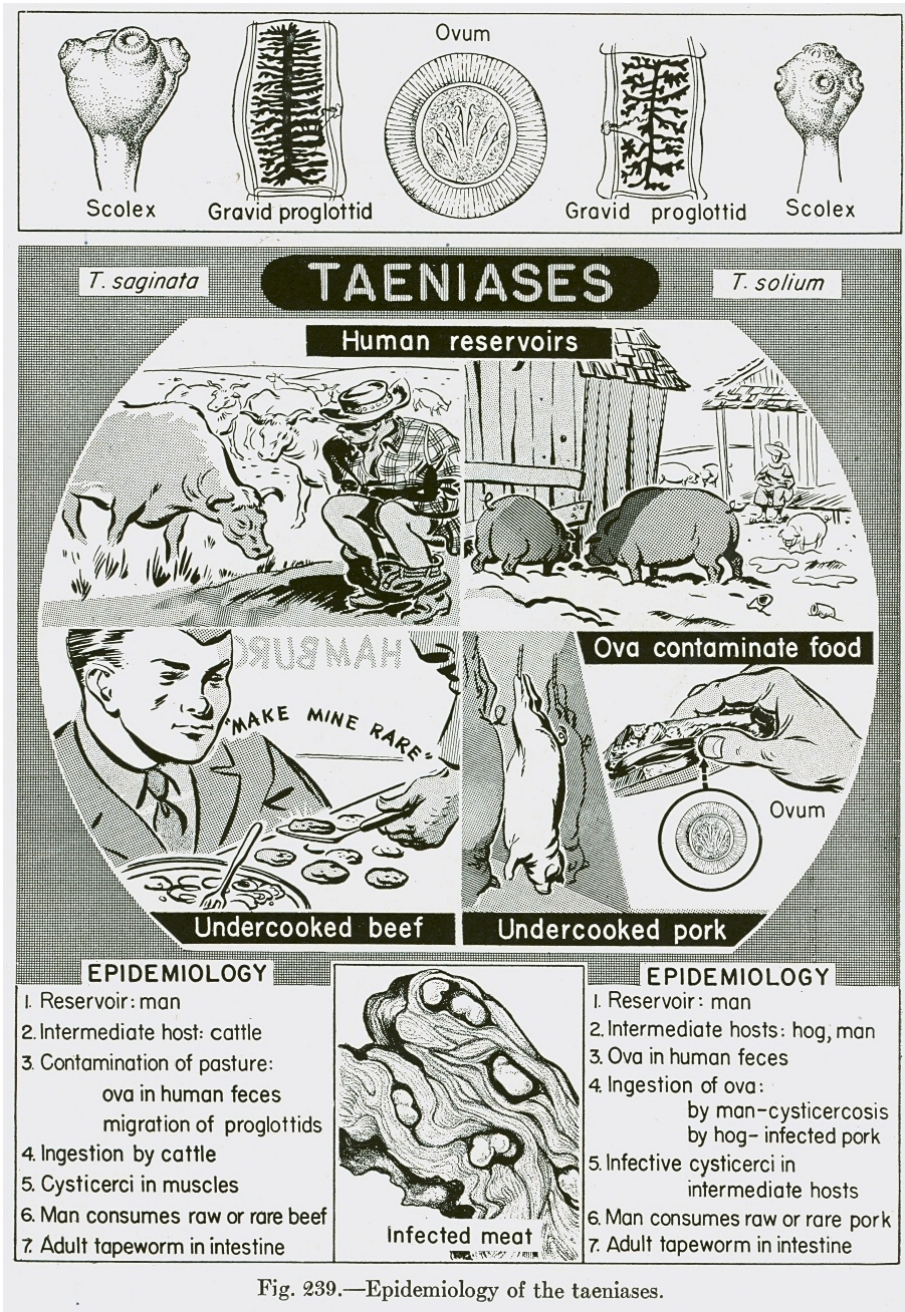


Fig. 239.—Epidemiology of the taeniases.

(Courtesy of A Manual of Tropical Medicine by Thomas T. Mackie, George W. Hunter III, and C Brooke Worth, 1945 by W. B. Saunders Company, Philadelphia)

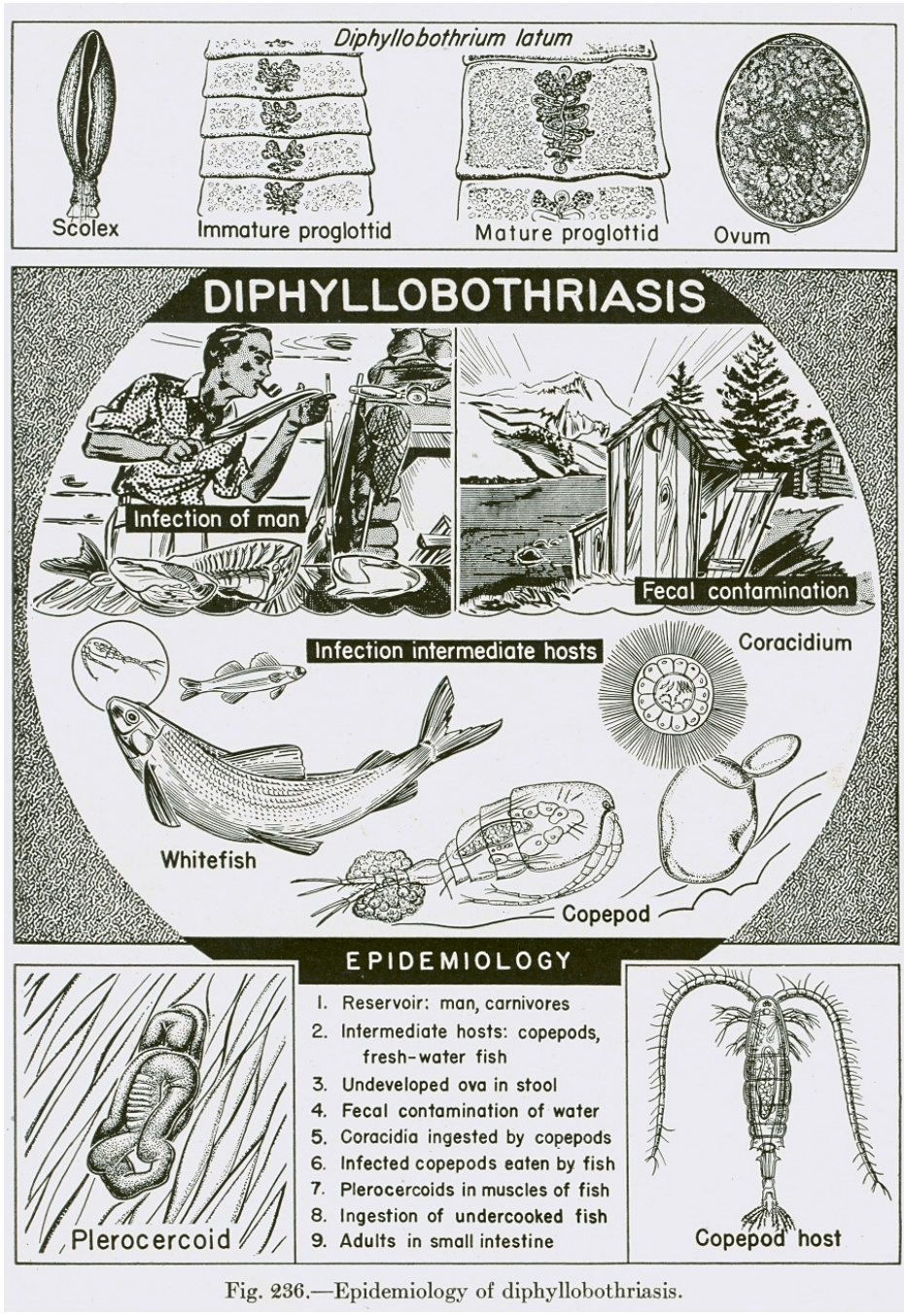


Fig. 236.—Epidemiology of diphyllobothriasis.

(Courtesy of A Manual of Tropical Medicine by Thomas T. Mackie, George W. Hunter III, and C Brooke Worth, 1945 by W. B. Saunders Company, Philadelphia)

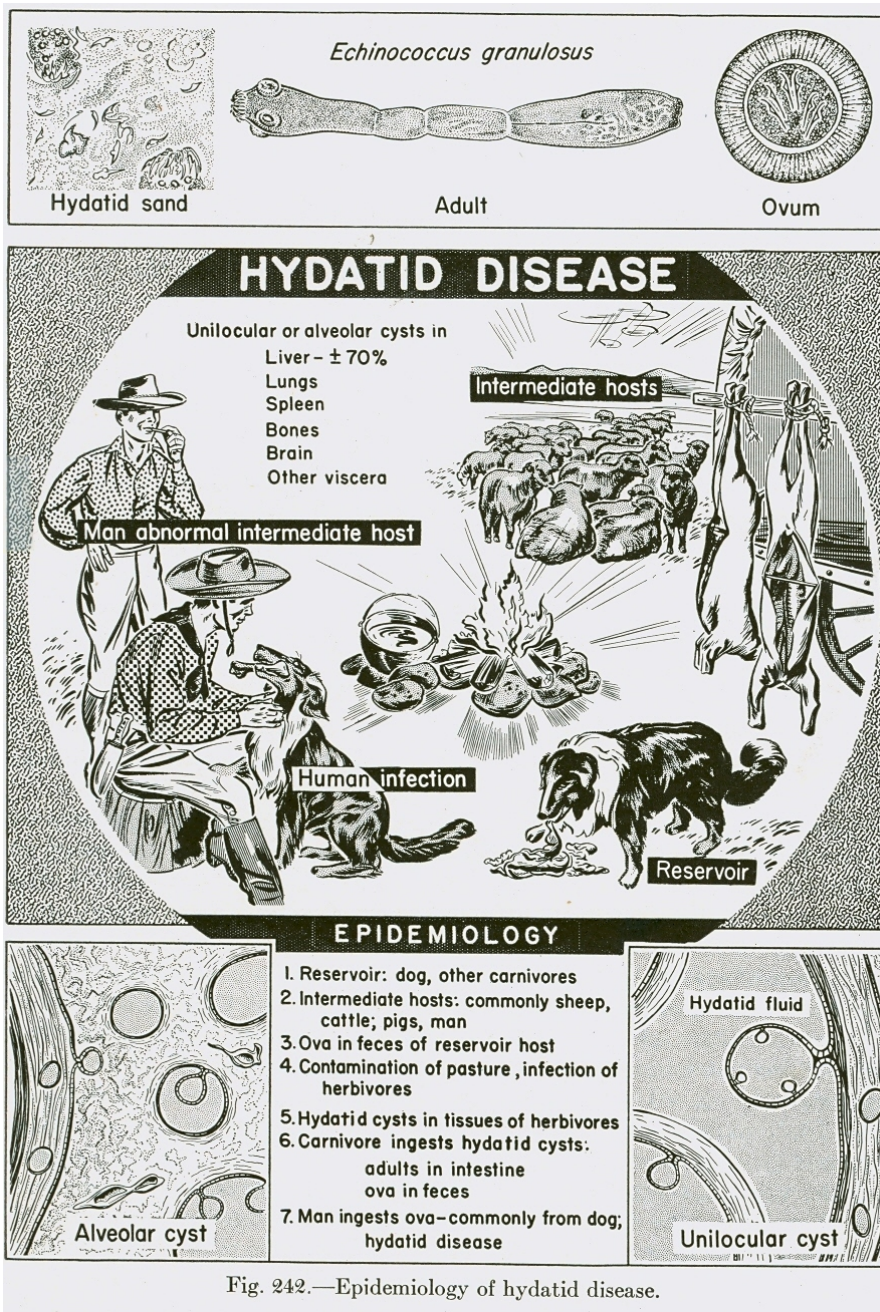


Fig. 242.—Epidemiology of hydatid disease.

(Courtesy of A Manual of Tropical Medicine by Thomas T. Mackie, George W. Hunter III, and C Brooke Worth, 1945 by W. B. Saunders Company, Philadelphia)

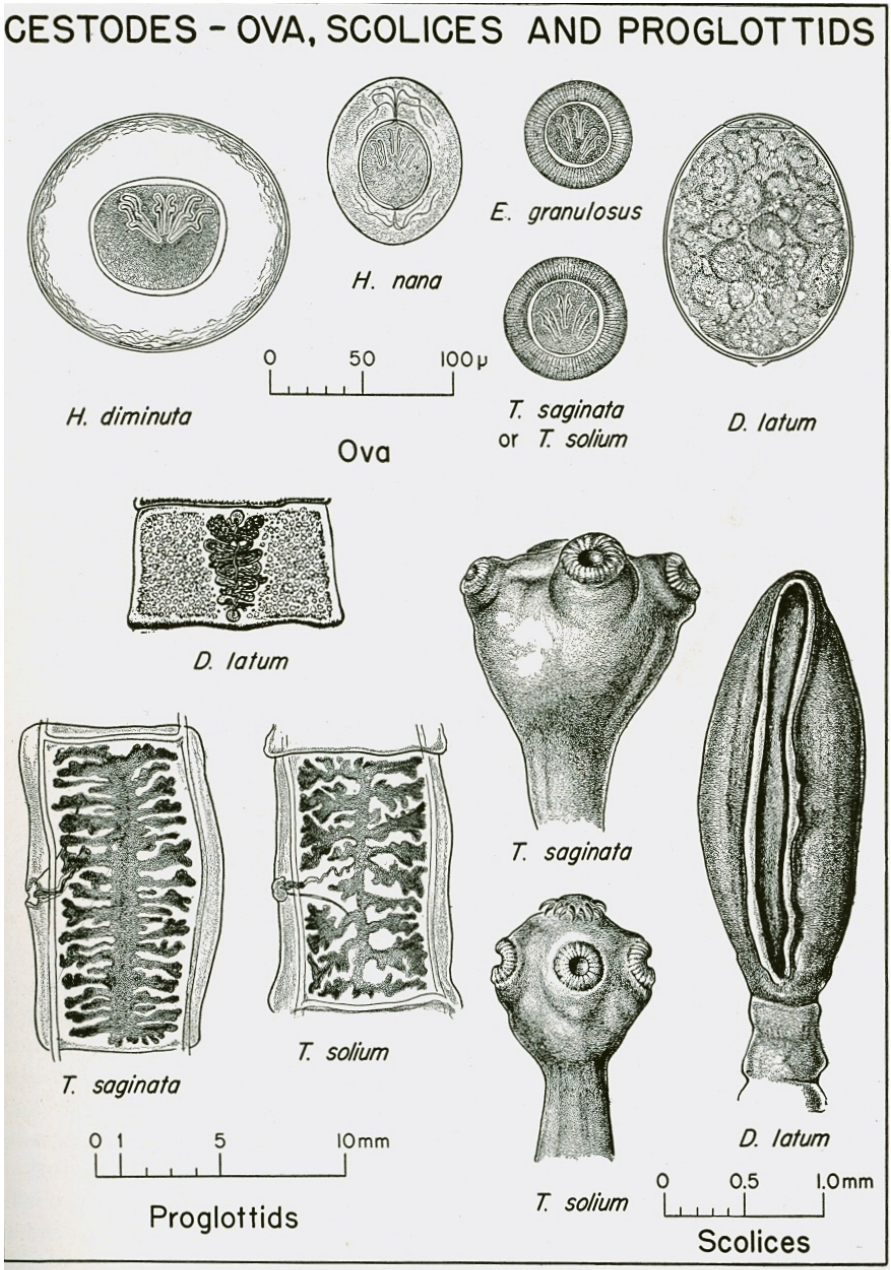
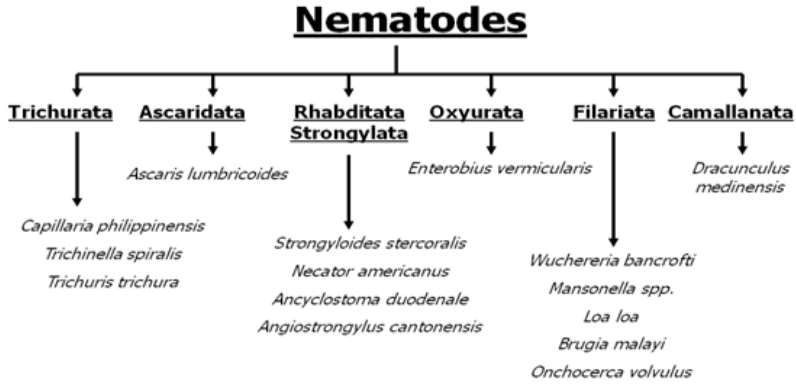


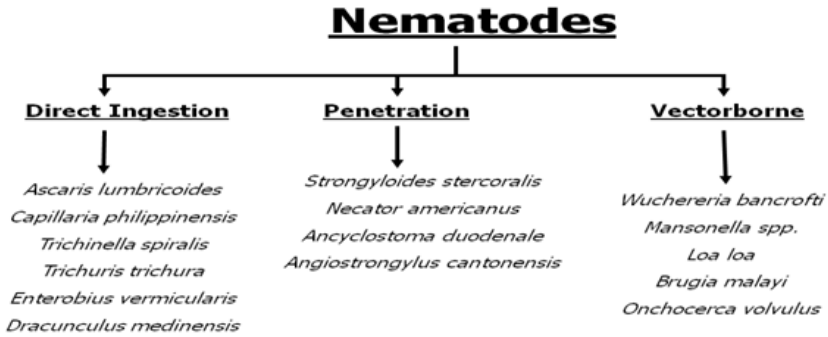
Fig. 232.—Ova, scolices and gravid proglottids of some tapeworms of man.

(Courtesy of A Manual of Tropical Medicine by Thomas T. Mackie, George W. Hunter III, and C Brooke Worth, 1945 by W. B. Saunders Company, Philadelphia)

The second group to be examined will be the roundworms (or Nematodes). The relationship of various groups within this major division is illustrated below:



Another way to look at the Nematodes is how they are transmitted:



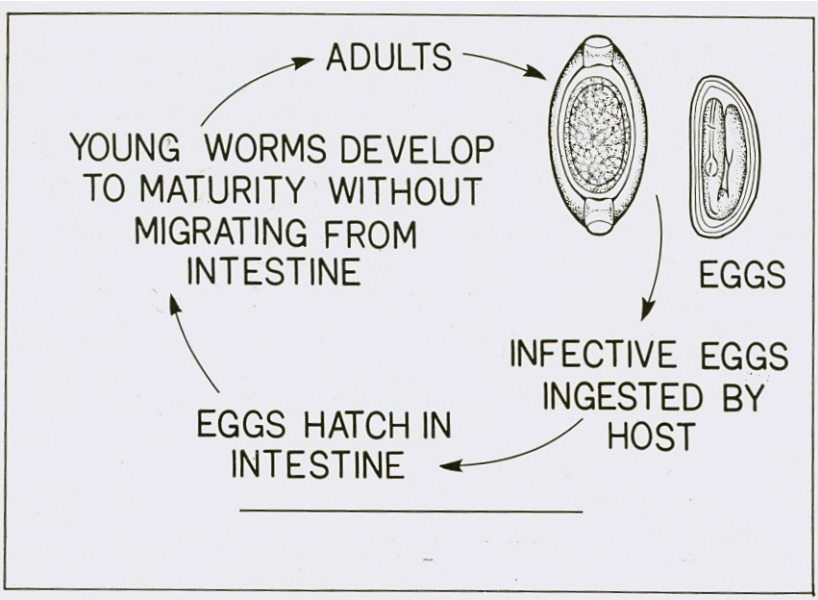


Fig. 158.—Nematode cycle—direct type.

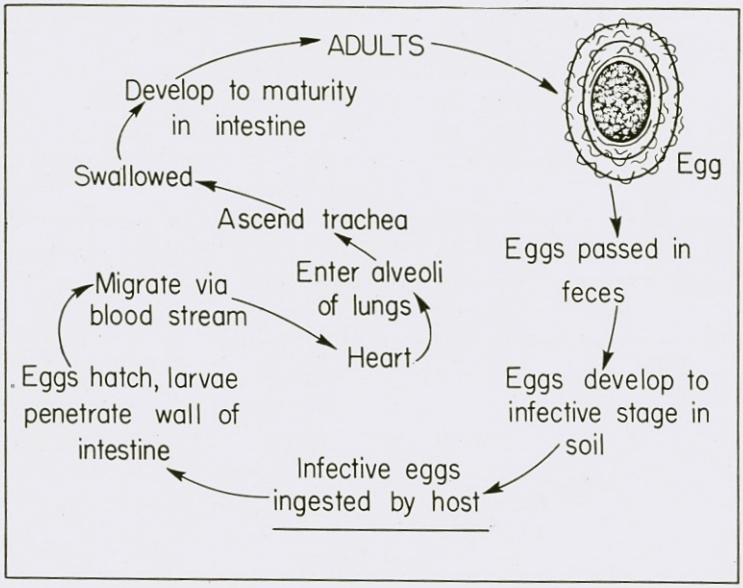


Fig. 159.—Nematode cycle—*Ascaris* type.

(Courtesy of A Manual of Tropical Medicine by Thomas T. Mackie, George W. Hunter III, and C Brooke Worth, 1945 by W. B. Saunders Company, Philadelphia)

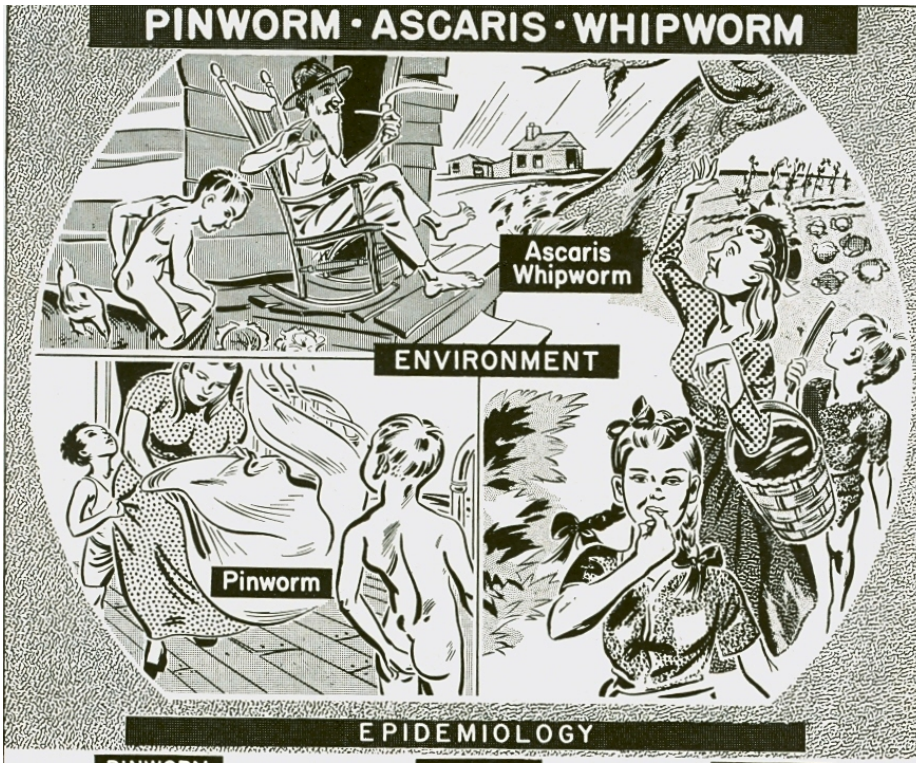


Fig. 164.—Epidemiology of pinworm, whipworm and ascaris infections.

(Courtesy of A Manual of Tropical Medicine by Thomas T. Mackie, George W. Hunter III, and C Brooke Worth, 1945 by W. B. Saunders Company, Philadelphia)

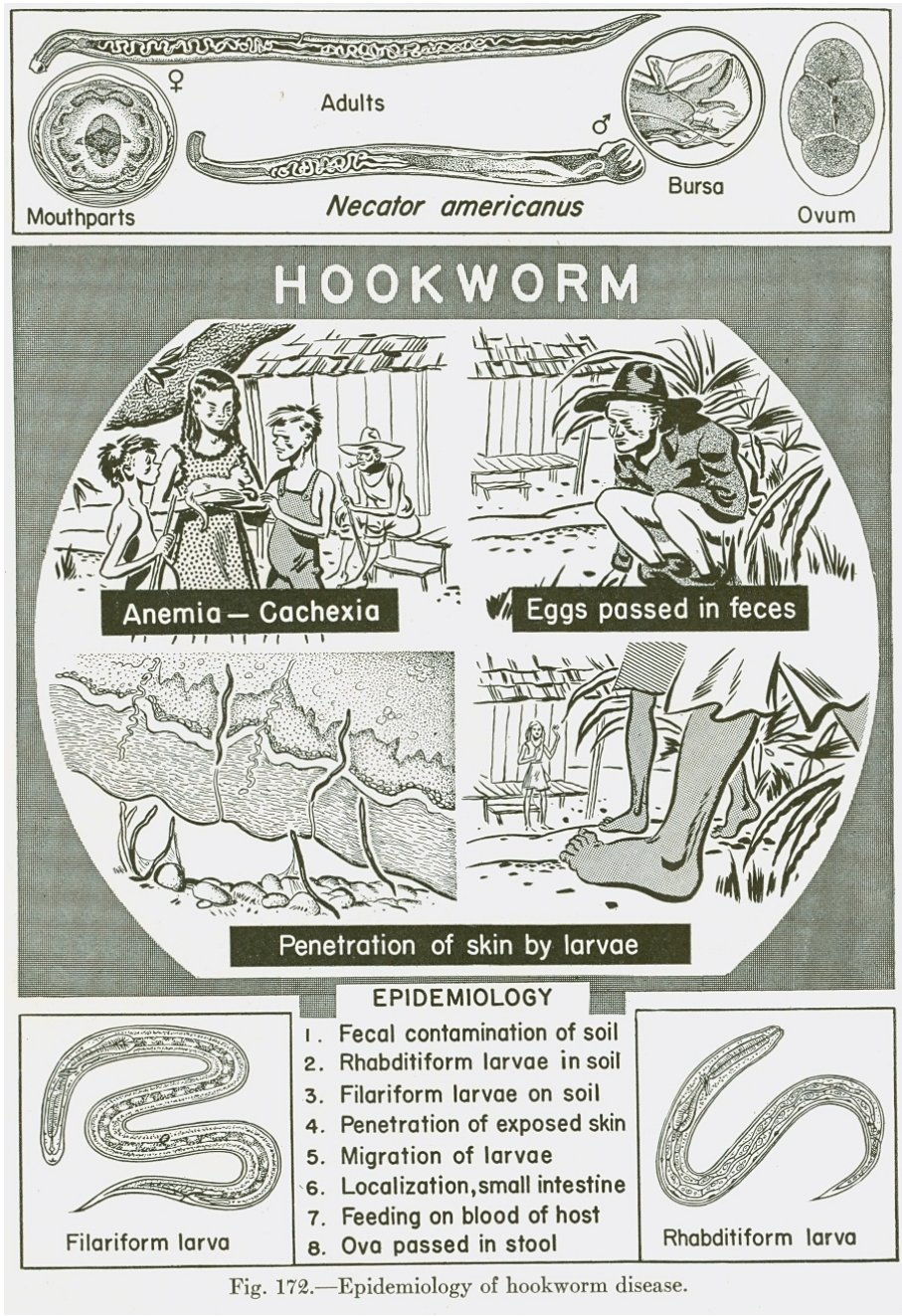


Fig. 172.—Epidemiology of hookworm disease.

(Courtesy of A Manual of Tropical Medicine by Thomas T. Mackie, George W. Hunter III, and C Brooke Worth, 1945 by W. B. Saunders Company, Philadelphia)

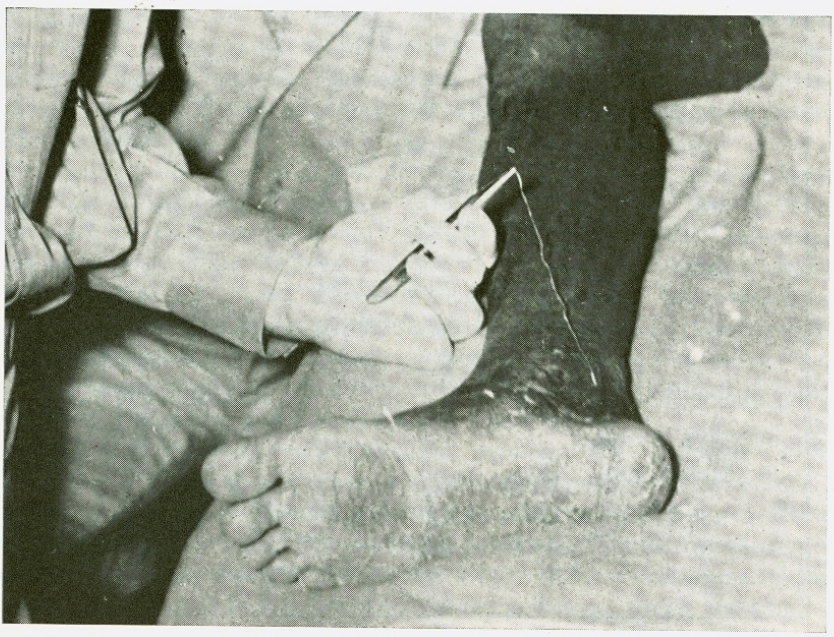
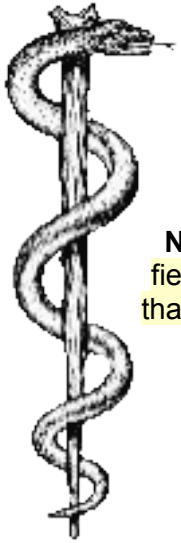


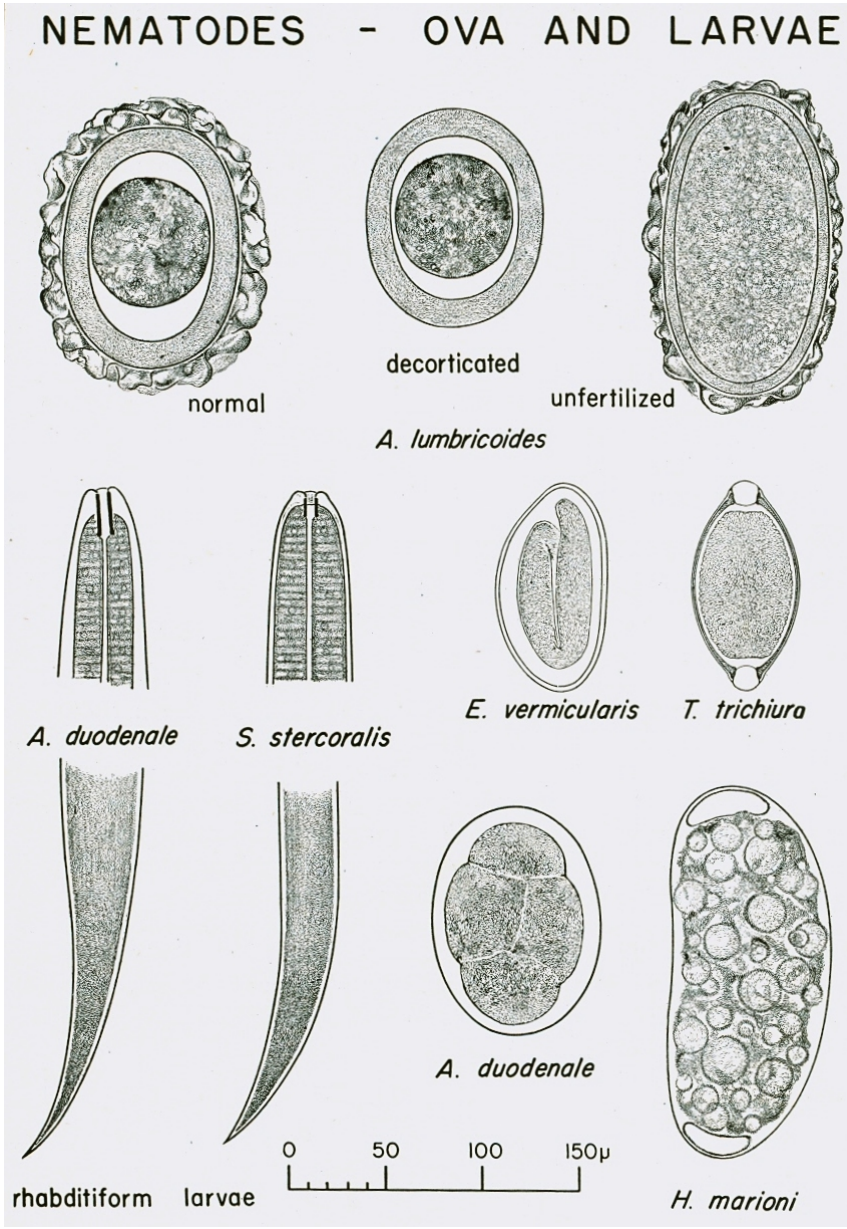
Fig. 202.—*Dracunculus medinensis* partially extracted. (Courtesy of Maj. J. M. Hulsey, Jr., M.C., A.U.S., through Lt. Col. Hardy A. Kemp, M.C., A.U.S., Army Medical School.)

(Courtesy of A Manual of Tropical Medicine by Thomas T. Mackie, George W. Hunter III, and C Brooke Worth, 1945 by W. B. Saunders Company, Philadelphia)



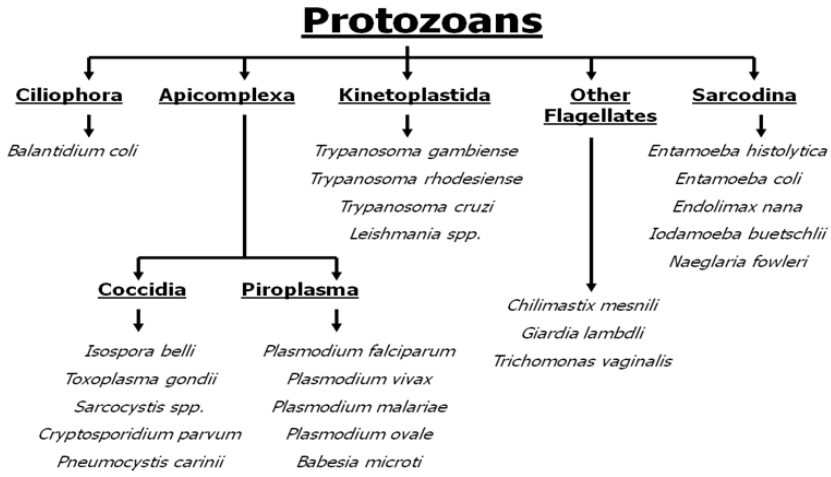
Numbers 21:8 And the LORD said unto Moses, Make thee a fiery serpent, and set it upon a pole: and it shall come to pass, that every one that is bitten, when he looketh upon it, shall live.

Rod of Asclepius



(Courtesy of A Manual of Tropical Medicine by Thomas T. Mackie, George W. Hunter III, and C Brooke Worth, 1945 by W. B. Saunders Company, Philadelphia)

The last group to be examined will be the single-celled organisms (or Protozoans). The relationship of various groups within this major division is illustrated below:



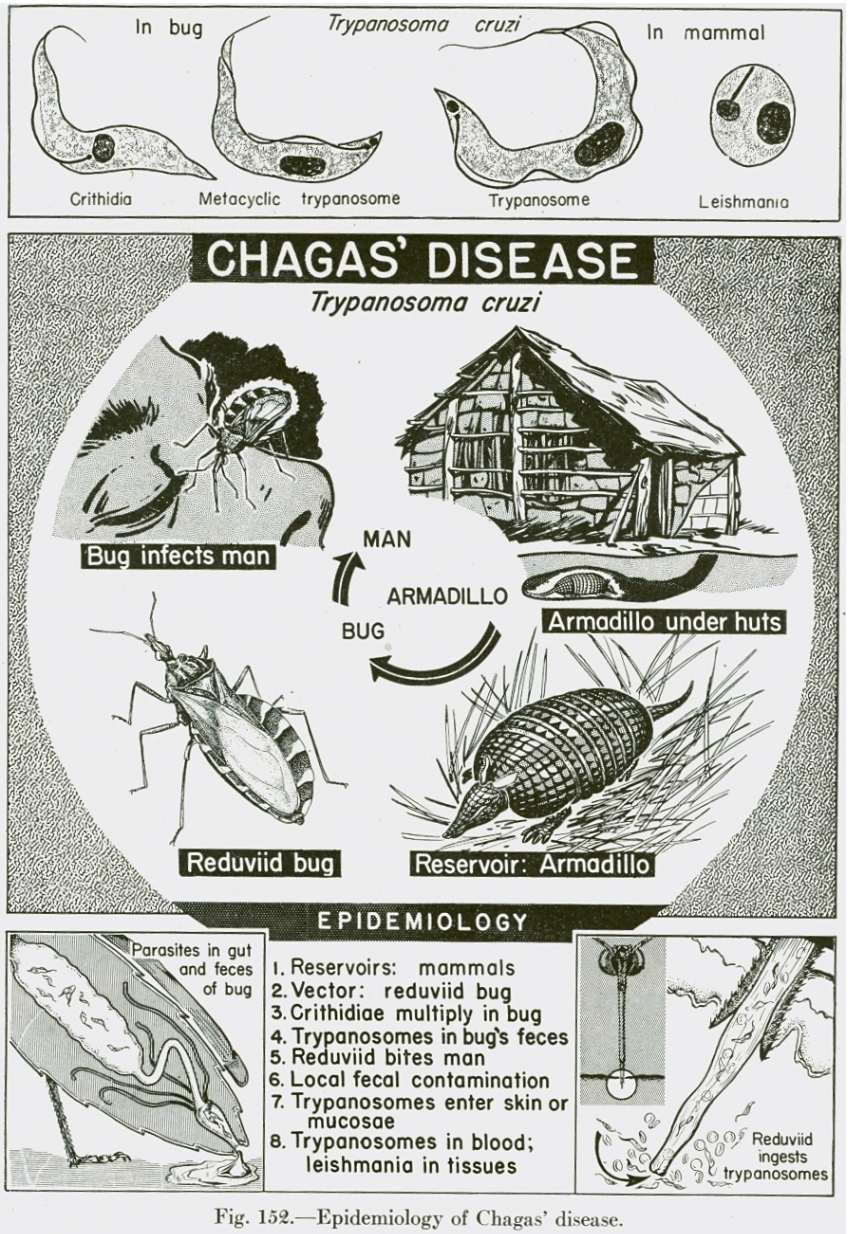


Fig. 152.—Epidemiology of Chagas' disease.

(Courtesy of A Manual of Tropical Medicine by Thomas T. Mackie, George W. Hunter III, and C Brooke Worth, 1945 by W. B. Saunders Company, Philadelphia)

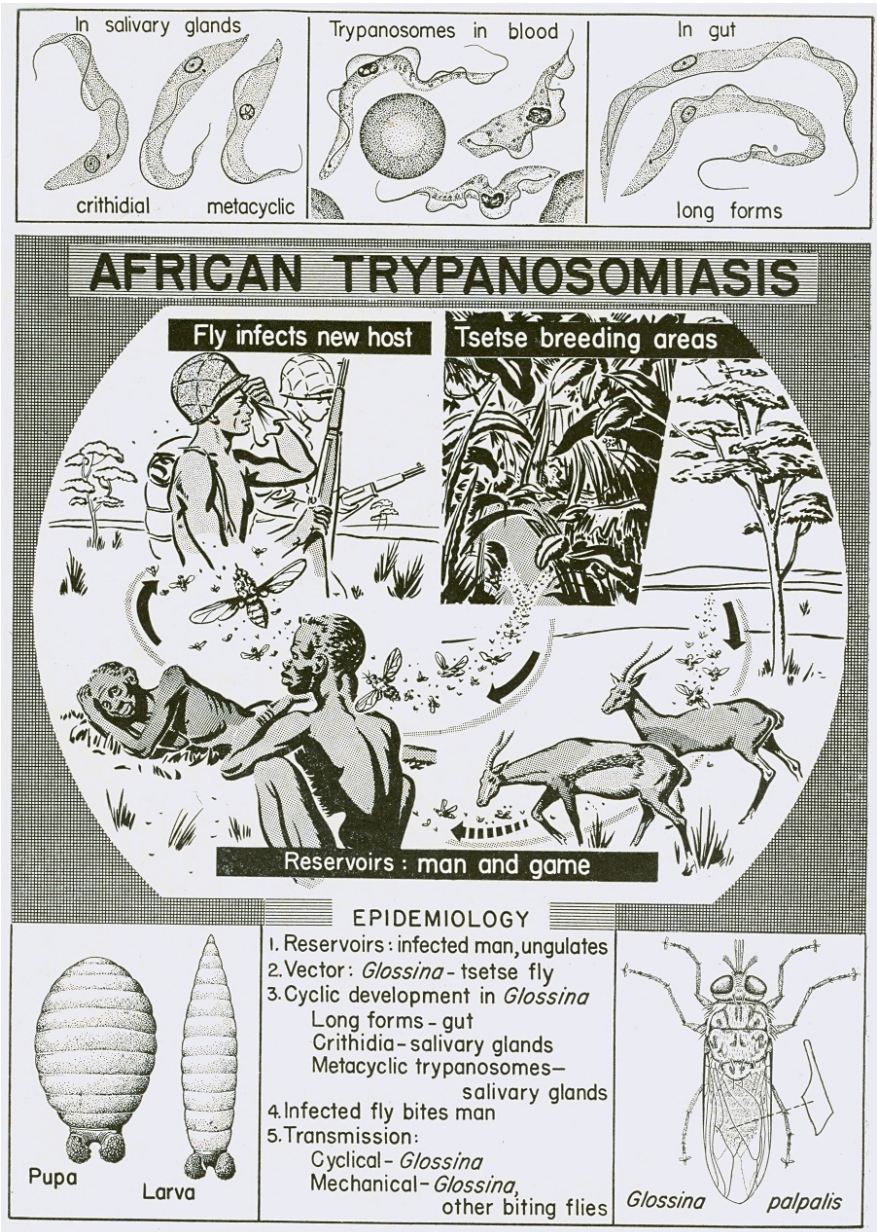


Fig. 146.—Epidemiology of African trypanosomiasis.

(Courtesy of A Manual of Tropical Medicine by Thomas T. Mackie, George W. Hunter III, and C Brooke Worth, 1945 by W. B. Saunders Company, Philadelphia)

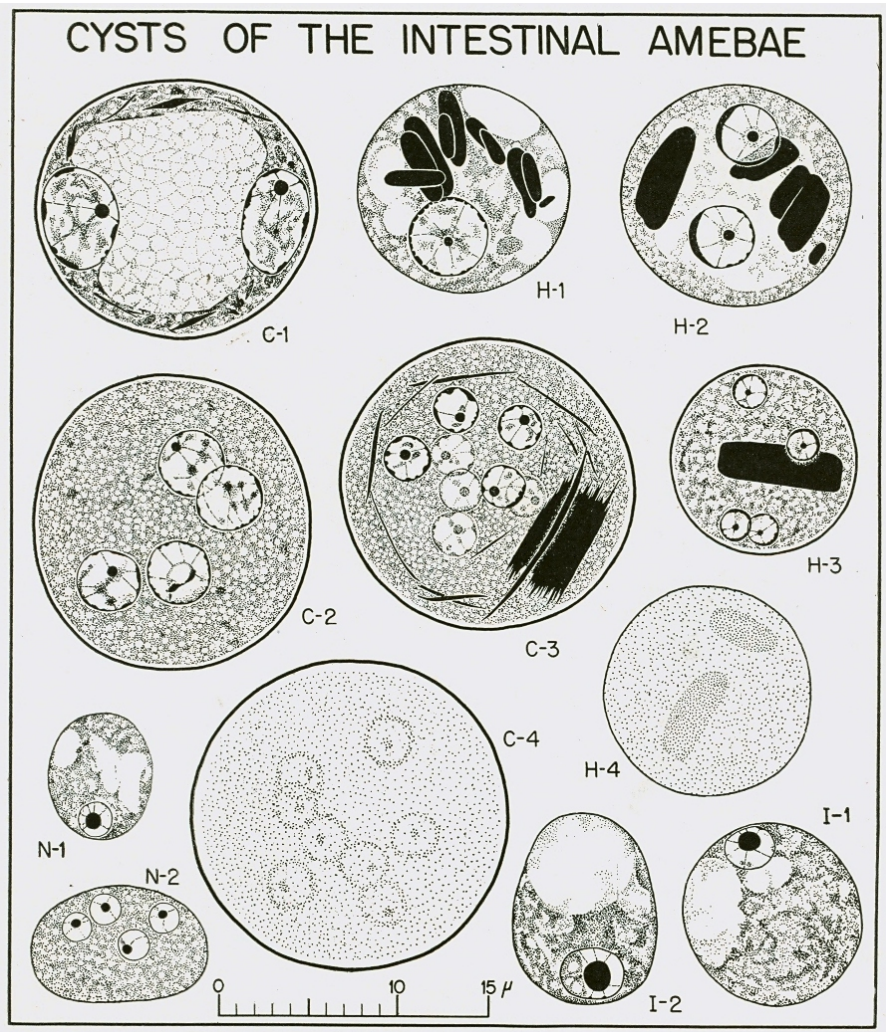


Fig. 99.—C1: Iron-hematoxylin stained binucleate cyst of *Endamoeba coli*. C2: Iron-hematoxylin stained quadrinucleate cyst of *E. coli*. C3: Iron-hematoxylin stained mature cyst of *E. coli*. H1: Iron-hematoxylin stained uninucleate cyst of *E. histolytica*. H2: Iron-hematoxylin stained binucleate cyst of *E. histolytica*. H3: Iron-hematoxylin stained mature cyst of *E. histolytica*. N1: Iron-hematoxylin stained uninucleate cyst of *Endolimax nana*. N2: Iron-hematoxylin stained mature cysts of *E. nana*. I1, I2: Iron-hematoxylin stained mature cysts of *Iodamoeba bütschlii*. C4: Unstained mature cyst of *E. coli*. H4: Unstained mature cyst of *E. histolytica*.

(Courtesy of A Manual of Tropical Medicine by Thomas T. Mackie, George W. Hunter III, and C Brooke Worth, 1945 by W. B. Saunders Company, Philadelphia)

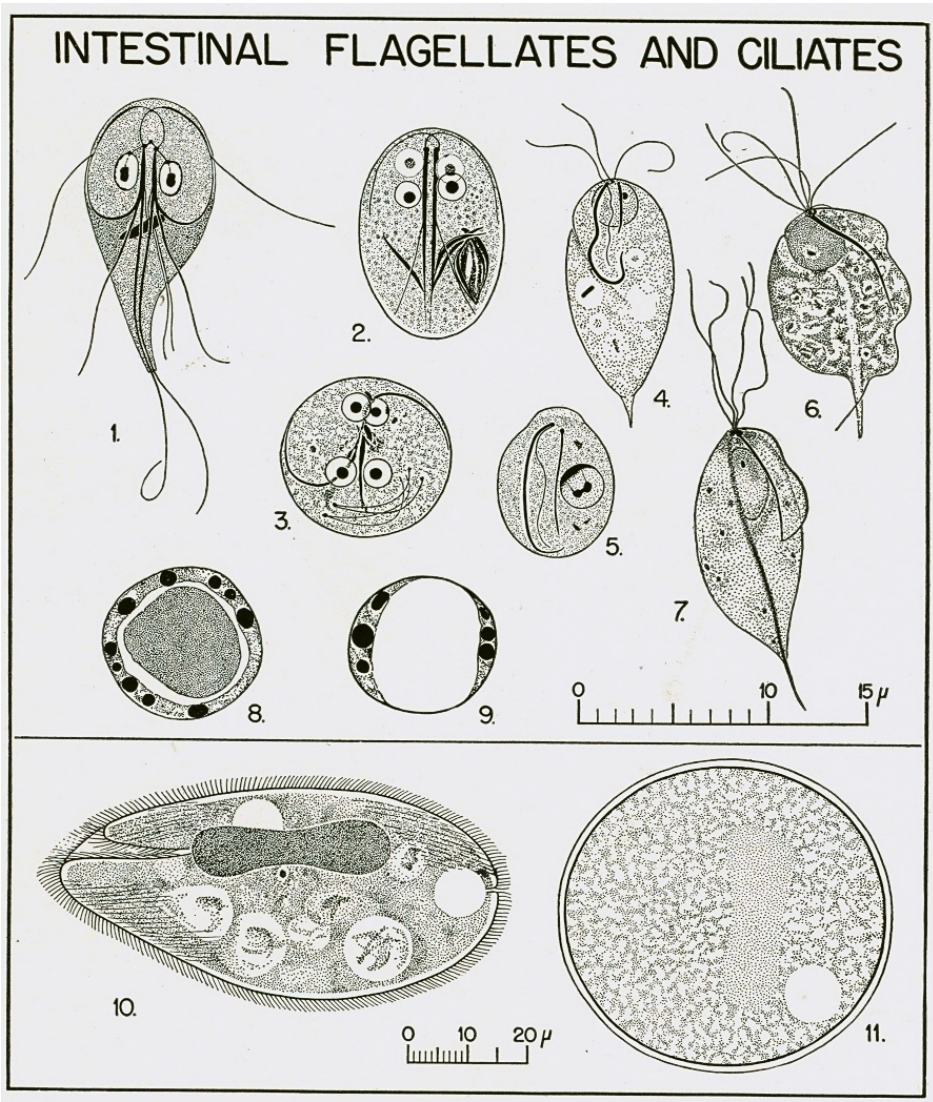


Fig. 100.—1. Iron-hematoxylin stained trophozoite of *Giardia lamblia*. 2. Iron-hematoxylin stained cyst of *G. lamblia*. 3. Iron-hematoxylin stained cyst of *G. lamblia* end-view. 4. Iron-hematoxylin stained trophozoite of *Chilomastix mesnili*. 5. Iron-hematoxylin stained cyst of *C. mesnili*. 6. Iron-hematoxylin stained trophozoite of *Trichomonas hominis*. 7. Iron-hematoxylin stained trophozoite of *T. vaginalis*. 8. Iron-hematoxylin stained *Blastocystis hominis*. 9. Unstained *B. hominis*. 10. Trophozoite of *Balantidium coli*. 11. Unstained cyst of *B. coli*.

(Courtesy of A Manual of Tropical Medicine by Thomas T. Mackie, George W. Hunter III, and C Brooke Worth, 1945 by W. B. Saunders Company, Philadelphia)

Entomology

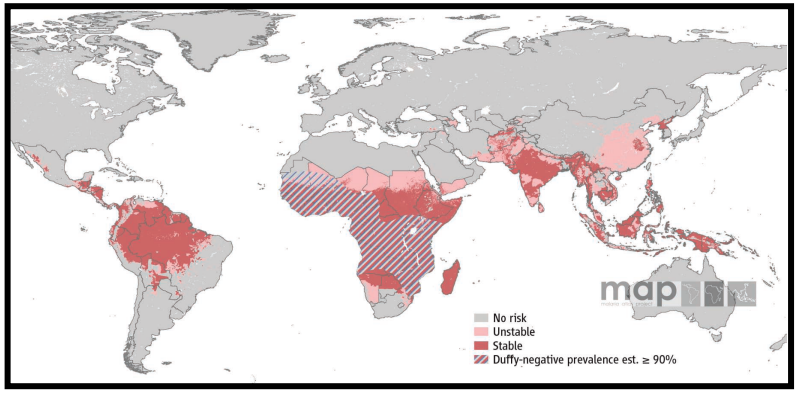
Mosquito-borne diseases. Mosquitoes are the most important group of vectors of human diseases. Throughout recorded history they have transmitted hundreds of different pathogens of humans, including: protozoans, viruses, and parasites that kill millions of people a year even today and debilitate hundreds of millions more. An example of some major pathogen groups is detailed briefly below. Websites that contain details about mosquito-borne human diseases, their vectors, distributions and prevention include:

1. Malaria

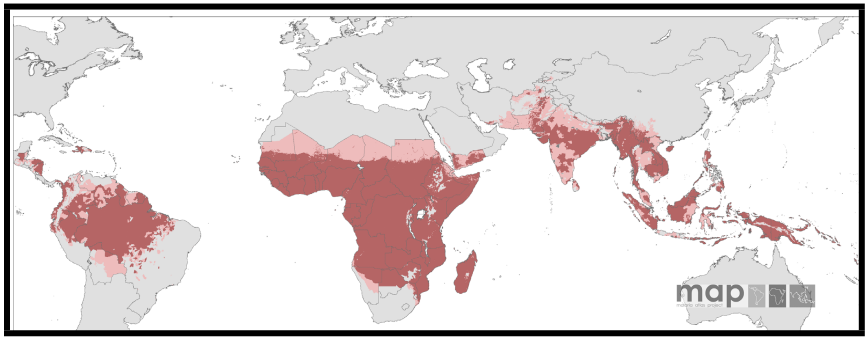
Malaria transmission is endemic in many tropical regions around the world, and transmission may occur nearly year round in many tropical countries, except at higher elevations (>500 m). The World Health Organization (WHO) estimated in 2009 that there are >300 Million new cases of malaria each year and that >1.5 Million of those are fatal each year with most of those cases and fatalities occurring in sub-Saharan Africa, India, and Southeast Asia. Vector mosquito activity and related intensity of transmission increase and decrease based on rainfall timing and patterns and also vary with the particular vector species, but are usually most intense during or shortly after peaks of rainfall in warm months. Strains of *Plasmodium falciparium* and *P. vivax* resistant to chemoprophylactic drugs have been reported to be present in some countries.

Adult females of at least 60 species of *Anopheles*, worldwide, have been proven capable of spreading the four species *Plasmodium* that cause human malarias. Many of these are species complexes and are still not well studied. Insecticide resistance is not regularly monitored in many countries, nor well reported. Historically, many countries have reported resistance or tolerance by several species of vector mosquitoes to commonly used insecticides.

Anyone going into a country or region where malaria is known or reported to be present should seek preventive and prophylactic advice (anti-malarial materials and dose guidance) from their higher medical authorities. They may also wish to do a search beforehand on websites of the WHO (www.who.int), the U.S. CDC (www.cdc.gov), or similar travelers' health sites, for their latest reported status and currently suggested chemoprophylactic drugs (and respective doses). Malaria may be a significant threat to either short or long term military operations in every place where it is endemic or has been recently reported to be actively transmitted. Appropriate personal protective techniques should be used routinely to help prevent infection by any vector-borne disease(s) wherever you may be in the world.

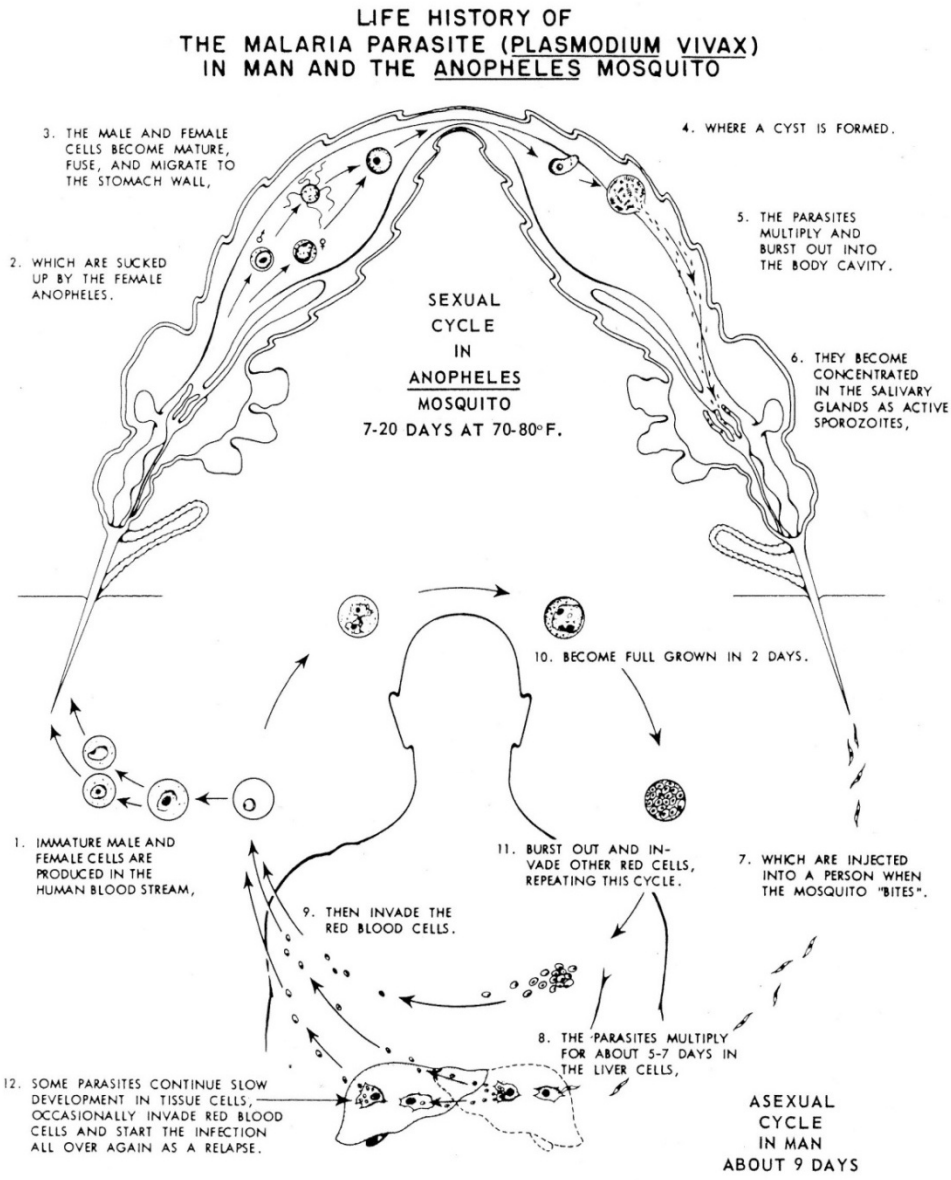


Areas at risk for *P. vivax* transmission - 2009



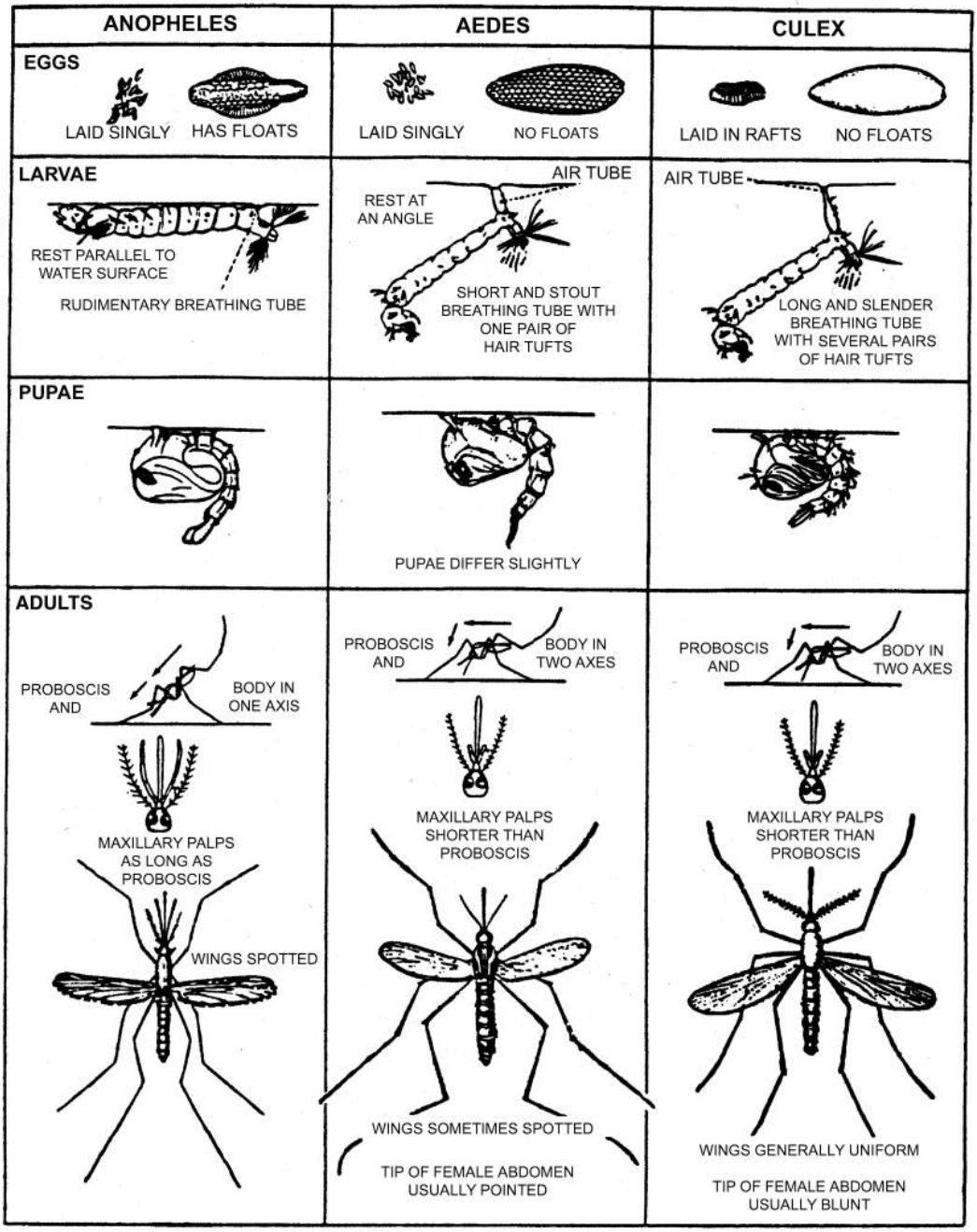
***P. falciparum* endemicity – 2009**

Malaria Disease Cycle(s)



1966 – U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE, PUBLIC HEALTH SERVICE
Communicable Disease Center, Atlanta, Georgia

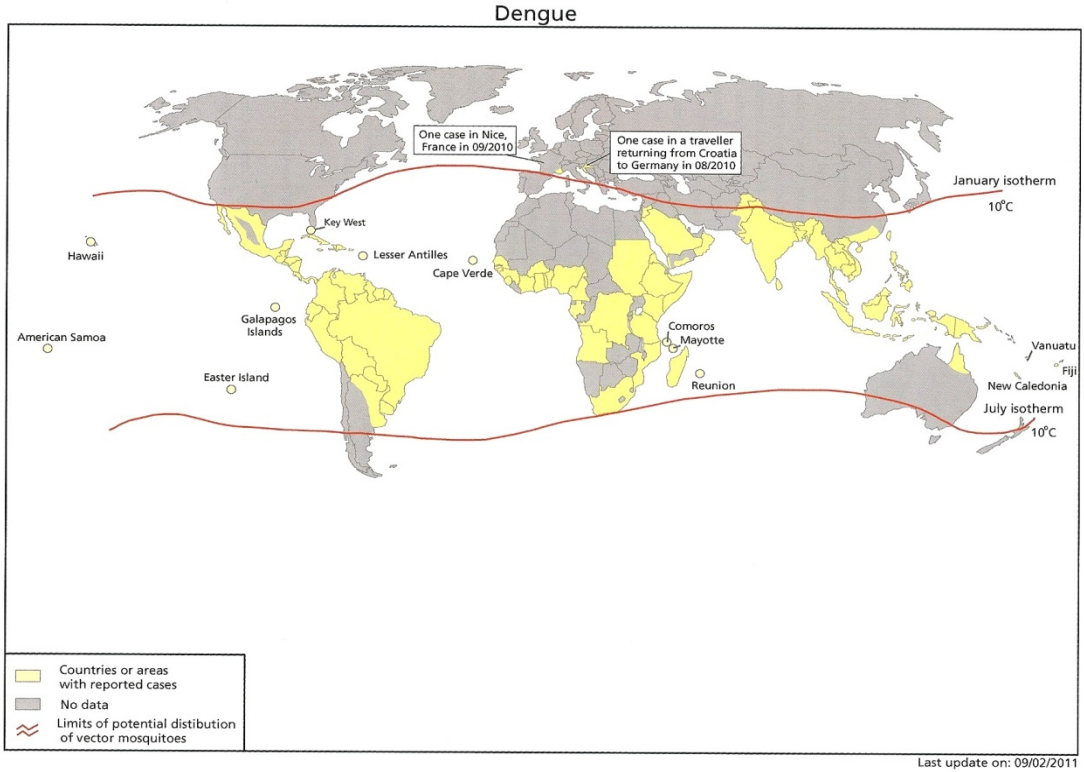
Comparison of characteristics of three major mosquito genera.



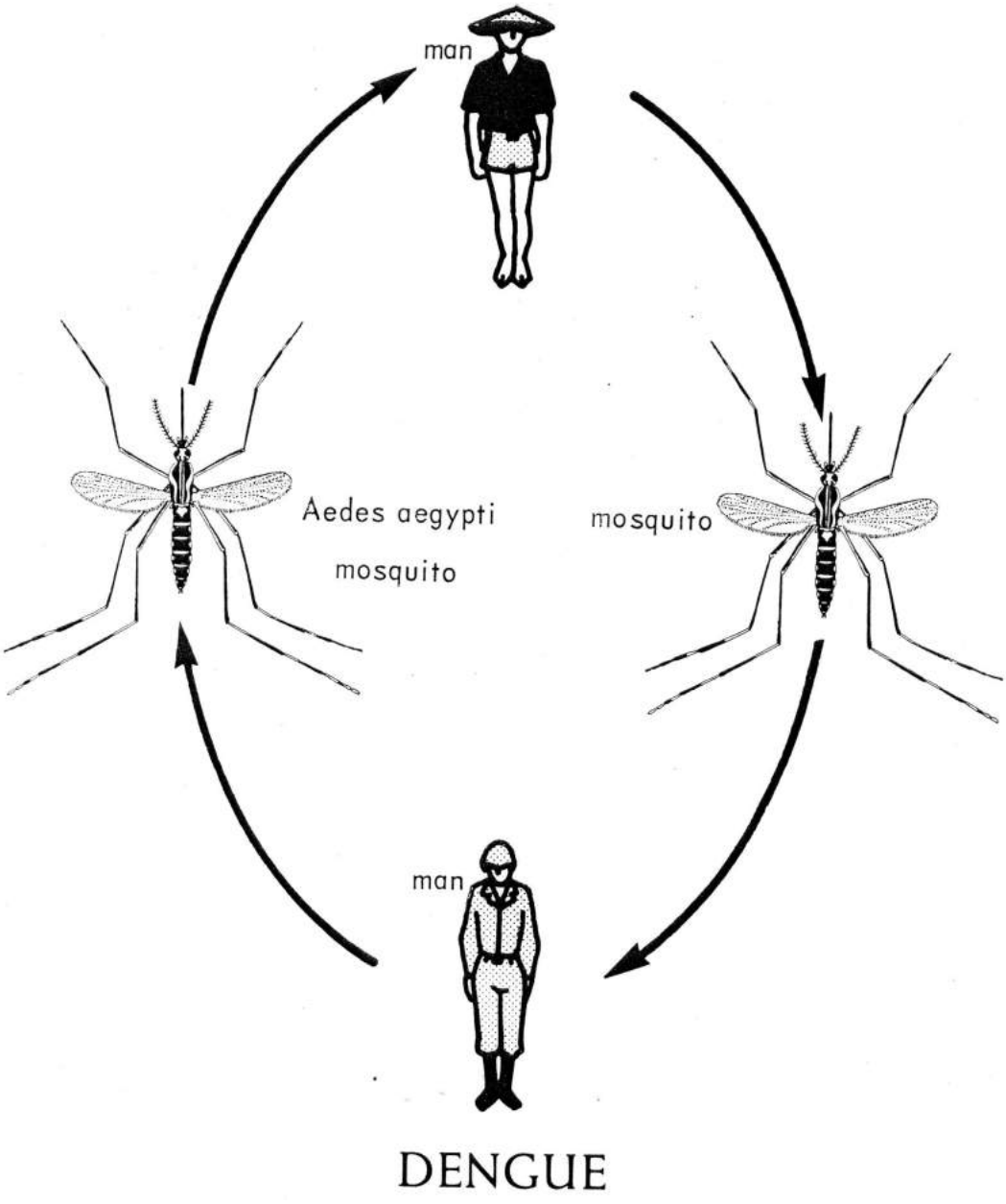
2. Dengue

Dengue virus (**DENV**) is widespread and essentially endemic throughout many tropical areas of the world. Periodic outbreaks of all four strains (serotypes DENV-1, -2, -3, & -4) occur and tend to go through poorly defined cycles of transmission at roughly 10-15 year intervals. Both **dengue hemorrhagic fever (DHF)** and **dengue shock syndrome (DSS)** have been reported currently or very recently from a number of tropical countries. Populations of the primary vector, *Aedes aegypti*, have greatly increased due partly to rapid and uncontrolled urbanization in much of Southeast Asia. *Aedes albopictus* is an important vector in peri-urban and rural areas of some regions and a cold-hardy strain of this species has spread from Northern Asia to the central U.S. and Brazil, and it was recently reported as established in northern Europe (i.e., the Netherlands). Dengue is a debilitating disease that would be a significant threat to military forces and there is no effective preventive vaccine or curative treatment, only supportive care (for current specifics see Heinemann 2009, and search the U.S. CDC and the WHO websites).

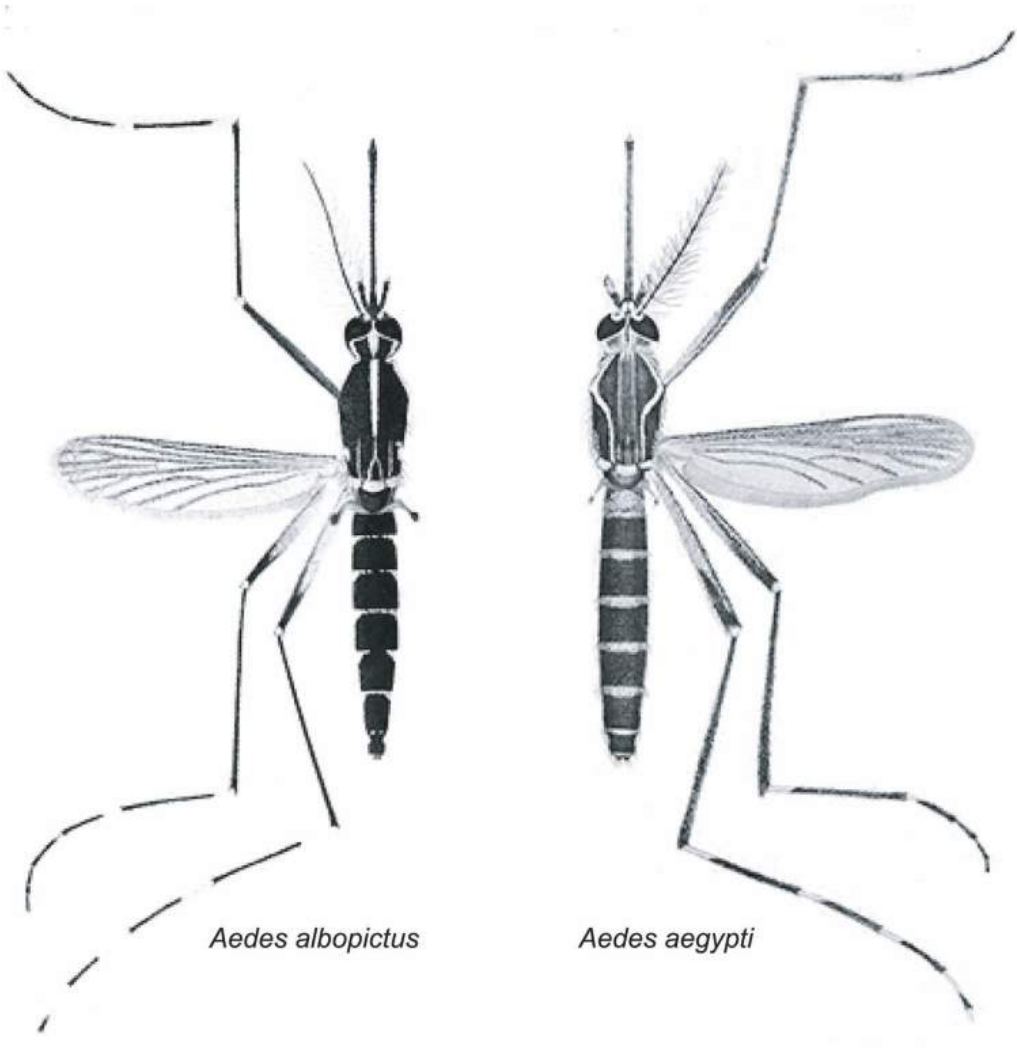
Distribution of Dengue:



Dengue Disease Cycle (an example of an arbovirus):



Some Vectors of Dengue: Comparison of Adult Female *Aedes aegypti* vs. *Aedes albopictus*.

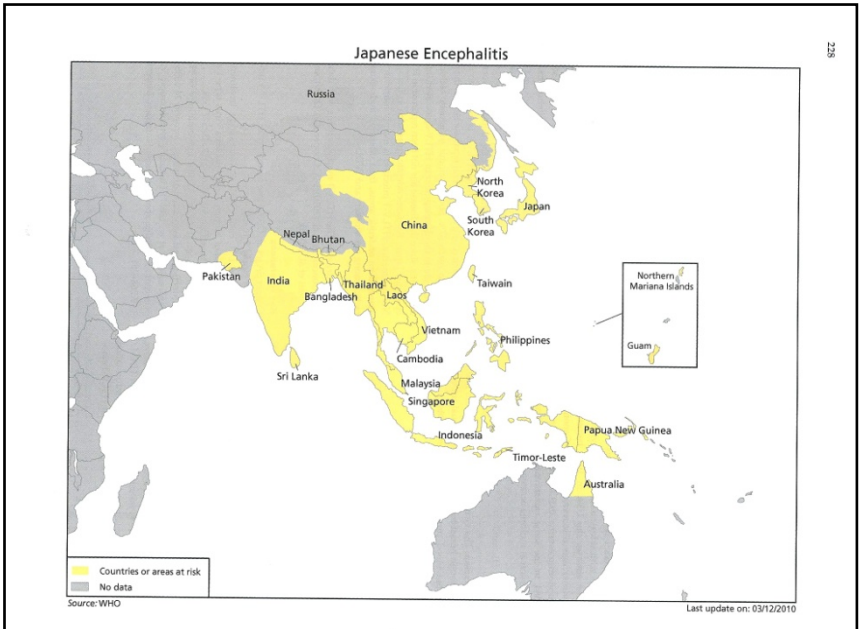


3. Chikungunya virus (CHIKV)

Chikungunya (CHIKV) virus is endemic to Africa and Asia and is transmitted by the same mosquitoes as DENV, *Ae. aegypti* and *Ae. albopictus*. CHIKV causes an illness with symptoms similar to dengue fever. CHIKV manifests itself with an acute febrile phase of the illness lasting only two to five days, followed by a prolonged arthralgic disease that affects the joints of the extremities. The pain associated with CHIKV infection of the joints persists for weeks or months, or in some cases years.

4. Japanese encephalitis (JE)

Japanese encephalitis (JE) virus has caused widespread epidemics in Japan and the Republic of Korea and is endemic in Southeast Asia. The virus is maintained in nature by mosquitoes and non-human vertebrates, and man becomes accidentally involved. In temperate countries like Japan, the disease occurs in the warm weather; in the tropics it could occur during any season, although the risk is higher during and immediately after the rainy season when the mosquito population increases. *Culex tritaeniorhynchus*, a rice field breeding mosquito is the principal vector and feeds mainly on large animals and birds. Elsewhere in the area of distribution, *Cx. gelidus* (predominantly a pig biter) and *Cx. vishnui* group mosquitoes are also involved. Japanese encephalitis is predominantly a rural disease and in most of southeastern Asia, associated with rice cultivation and mosquitoes which breed in rice fields. Transmission is by bite. A licensed vaccine is available.

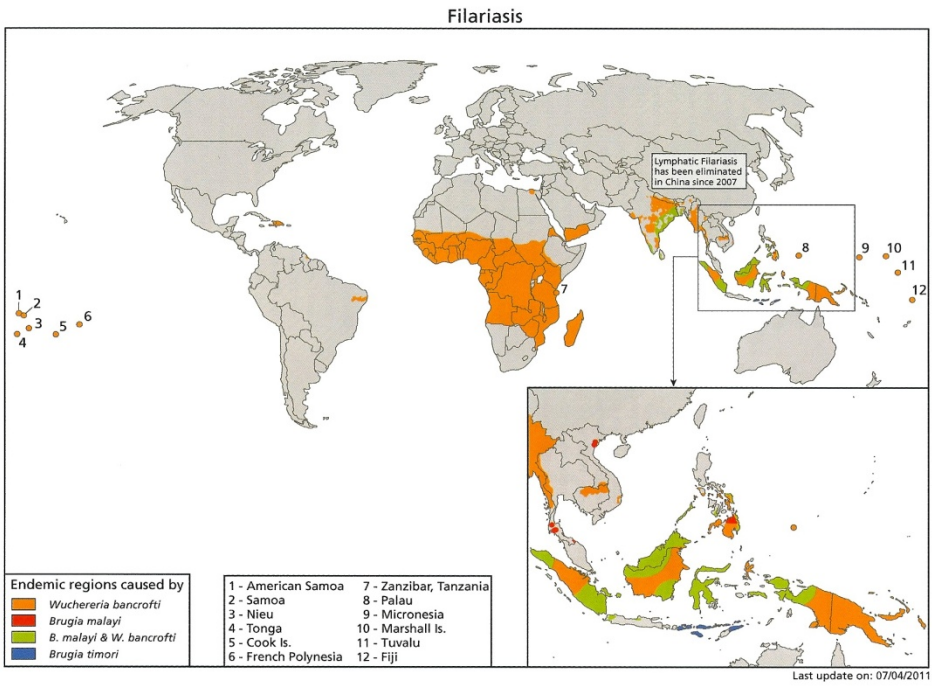


Distribution of reported FEV cases from Tiroumourougane1 et al 2002.

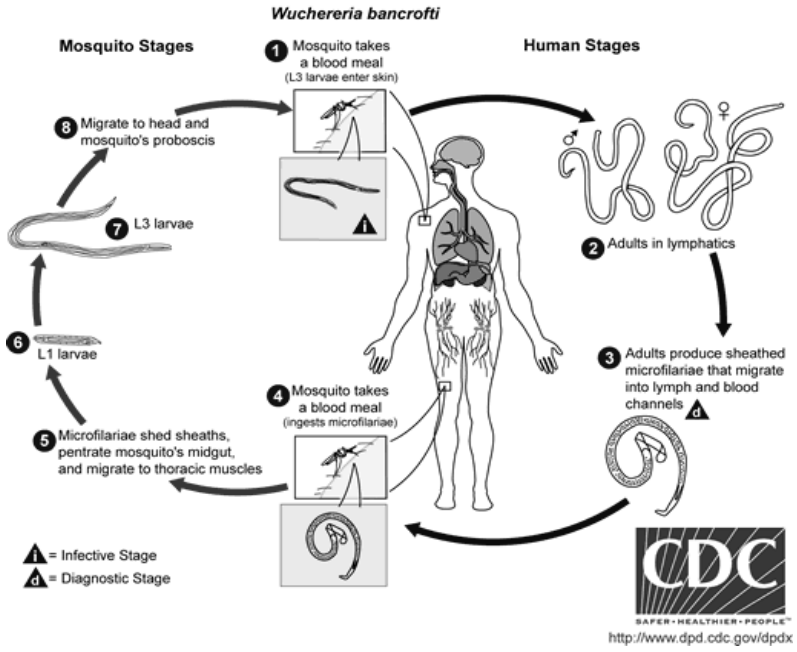
5. Filariasis

Bancroftian filariasis, caused by ***Wuchereria bancrofti***, and **Brugian filariasis**, caused by ***Brugia malayi***, are now lumped by the WHO as "**Lymphatic Filariasis**" **(LF)**. According to the WHO (2007), LF is currently endemic and widespread, and poses serious public health problems in Southeast Asia, South Asia, and sub-Saharan Africa. Mapping of the populations at risk and related programs of mass drug administration (MDA), using oral dosing with diethylcarbamazine citrate (DEC), are in progress in a number of countries, and seem to be reducing the levels of LF morbidity therein. The main vectors of Bancroftian filariasis, *Culex pallens* and *Cx. quinquefasciatus*, have become more abundant as breeding sites have been expanded due to increased urbanization and poor sanitation. Nocturnally periodic forms of *B. malayi* and *W. bancrofti* are also currently endemic in parts of Southeast Asia and some southwest Pacific islands. At least 16 different species of tropical area mosquitoes are reported to be effective vectors of filariases (LF), including several species of *Anopheles*.

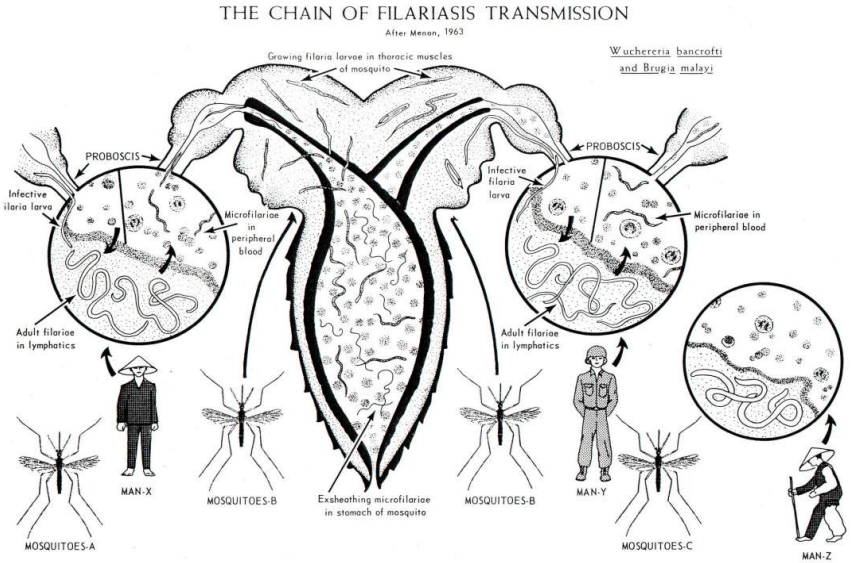
Distribution of Filariasis:



Filariasis disease cycle (Bancroftian):



Typical transmission cycles for *Wuchereria bancrofti* and *Brugia malayi*:



Explanatory notes: –
Infective vector mosquitoes (A) repeatedly inoculate Man "X" who becomes a symptomless microfilaria carrier in about a year or more; he infects numerous vector mosquitoes (B) which in turn inoculate Man "Y" who also becomes a microfilaria carrier and passes on the infection to still more vector mosquitoes (C).

A symptomless carrier (Man "X" or "Y"), continually exposed to further bites of infected vectors, may later develop filarial disease (Man "Z") with the onset of disease, his peripheral blood usually becomes free from microfilariae so that he is no longer a carrier.

Prepared by Harold George Scott, Ph.D., and Margery Bacon

Sand Fly-borne Diseases. One of the issues with sand fly borne diseases is that there are no vaccines or prophylactics available for them. For this reason vector control and personal protective measures are the only defenses against contracting these diseases. Sand flies are much smaller than most mosquitoes. Like mosquitoes only females take a blood meal and are capable of disease transmission. Unlike mosquitoes they do not have an aquatic phase to their life cycle. They are found in temperate and warmer environments, ranging from forests to deserts. The larvae feed on decaying organic matter from decaying vegetation to feces. Adult sand flies can be distinguished from most other blood-sucking arthropods including their related mosquitoes in that they hold their wings in a "V" shape rather than laying them flat against their abdomens. They also tend to "hop" after lighting on surfaces. When examining light trap collections their small size, hunched back, long legs, and the fine hairs (rather than scales) along with their wings held away from their bodies helps to separate them from other insects.

1. Leishmaniasis

Leishmaniasis is an infection of animals and humans caused by protozoa in the genus *Leishmania*. At least 23 *Leishmania* species cause leishmaniasis. There are three forms of the disease: cutaneous (CL), mucocutaneous (MCL) and visceral (VL). CL appears as a non-healing ulcer lasting months to years if untreated. MCL patients develop ulcerative or granulomatous (granular) lesions of the nasal, oral, and pharyngeal linings, which generally occur after or concurrent with CL lesions. VL, the most severe form of leishmaniasis with 95% mortality in untreated cases, is a chronic disease involving the liver and spleen.

Transmission occurs through the bite of infective female phlebotomine sand flies in the genera *Phlebotomus* (Old World) and *Lutzomyia* (New World). Generally, sand flies feed at dusk and during the evening; however, some species are opportunistic and will feed during the day if disturbed. The uninfected sand fly generally acquires the infection by feeding on a reservoir host. Reservoirs for leishmaniasis, which vary depending on location, include domestic dogs, rodents (including rats, hyraxes and gerbils), sloths, marsupials, and in some endemic areas, humans. It is reported that 90 species of *Lutzomyia* and at least 39 species of *Phlebotomus* feed on humans.

Protect Yourself from Leishmaniasis. Limit outdoor activity at dusk and during the evening when possible as this is when the sand fly is most active. If possible building should have window screens or other barriers to keep sand flies from entering. Sand flies bite in and outdoors; although generally nocturnal, they may feed during the day in buildings. Avoid the bites of sand flies by using protective clothing and insect repellents (See Personal Protective Measures in the Vector Management Section).

2. Sand Fly Fever

Sand Fly Fever is a *Phlebovirus*, (Bunyaviridae) with two major serotypes, Sicilian and Naples. It occurs in the Mediterranean, Mid-East and North Africa. A major vector is *Phlebotomus papatasi*; however, several other species of Phlebotomus also serve as vectors.

Female *Phlebotomus papatasi* Male *Lutzomyia longipalpis*

http://www.raywilsonbirdphotography.co.uk/Galleries/Invertebrates/000_invert_images/vector_images/2009-05-20_JS8Q8643-Lu_longipalpis.jpg

Control and Personal Protective Measures would be those used for other sand fly borne diseases such as Leishmaniasis.

Tick-borne Diseases. Tick-borne illnesses are caused by infection with a variety of pathogens, including rickettsia and other types of bacteria, viruses, and protozoa. Because ticks can harbor more than one disease-causing agent, patients can be infected with more than one pathogen at the same time, compounding the difficulty in diagnosis and treatment. The geographic range of many tick-borne diseases is not well known and symptoms are often similar to other bacterial or viral diseases. Prevention of tick bites should be stressed using methods described below.

Vector Surveillance and Suppression. Light traps can be used to collect night-biting mosquitoes, phlebotomine sand flies, and Culicoides. Not all species are attracted to light and adding carbon dioxide (CO2) to light traps (figure 1) increases the number of species and total numbers of females collected. Traps baited with animals or humans or placed around their habitations can be useful for determining risk in these areas and may also increase catch. (the use of humans as attractants may be subject to the requirements of formal human-use protocols). Adults can be collected from indoor and outdoor resting sites using a mechanical aspirator and flashlight. For mosquitoes systematic larval sampling with a long-handled white dipper provides information on species composition and population dynamics of species breeding locally that can be used to plan control measures. Larva sampling are generally not effective for sand flies.

Typical light trap used in the surveillance of night biting insects. These traps come in different formats but consist basically of a light and a fan which drives the insects into a collection jar or net when they get close to the light. Carbon dioxide can be added to the trap in the form dry ice or compressed gas cylinder.

Figure 1. Mosquito light trap.

Vector Management (Control)

1. Personal protective measures

Personal protective measures are the first line of defense against arthropod-borne disease and, in some cases, may be the only protection for deployed military personnel. Proper wearing of the uniform and appropriate use of repellents can provide high levels of protection against blood-sucking arthropods. The uniform fabric provides a significant mechanical barrier to mosquitoes and other blood-sucking insects. Therefore, the uniform should be worn to cover as much skin as possible if weather and physical activity permit.

Some newly developed repellents provide military personnel unprecedented levels of protection. An aerosol formulation of permethrin (**NSN 6840-01-278-1336**) can be applied to a uniform according to label directions, but not to the skin. This will impart both repellent and insecticidal properties to the uniform material that will be retained through numerous washings. An extended formulation lotion of N, N-diethyl-m-toluamide (DEET) (**NSN 6840-01-284-3982**) has

been developed to replace the older 2 oz. bottles of 75% deet in alcohol. This lotion contains 33% active ingredient. It is less irritating to the skin, has less odor and is generally more acceptable to users.

A properly worn Uniform (e.g., ACU) impregnated with permethrin, combined with use of extended duration DEET on exposed skin, has been demonstrated to provide nearly 100% protection against a variety of blood-sucking arthropods. This dual strategy is termed "**the DoD Arthropod Repellent System.**" In addition, permethrin may be applied to bednets, tents, and other field items as appropriate. Complete details regarding these and other personal protective measures are provided in TG 36, Personal Protective Techniques Against Insects and Other Arthropods of Military Significance (2009).

2. Physical Controls

Soldiers often do not have the option to avoid potential disease vector or their typical habitats, but physical controls may sometimes be possible. Covering structural openings (e.g., doors and windows) with screen fine enough to exclude particular vectors or biting pests may be very practical, especially in permanent or semi-permanent camps. Sealing cracks and joints in even temporary structures and vehicles whenever possible or covering such openings with netting (like a bed net, or part of an old one) might keep out a lot of filth flies that can spread food-borne diseases), as well as scorpions and similar crawling pests that can pose a health threat. The use of fly swatters or similar devices against individual invaders falls within this same category of control. Use a bed net while sleeping; **NSN 7210-00-266-9736 (Netting), NSN 7210-00-267-5641 (Poles)**. Because sand flies are small enough to pass through the mesh of the standard bed net, permethrin (aerosol spray) should be applied to netting. There is enough permethrin in one spray can to treat one uniform and a bed net.

3. Source Reduction

Eliminating standing water or treating it with non-toxic or minimally toxic (to humans) larvicides like *Bti* or an insect growth regulator (an IGR) within a 100-ft. radius of any point can greatly reduce the local breeding and short-range attraction of a number of mosquitoes, Phlebotomine sand flies, and certain other vectors and pests. Surface drainage, filling pot holes, and similar actions can also help reduce the numbers of a given vector or pest species that any

particular habitat can support. Removing trash, garbage and rubbish frequently could reduce the habitat for a number of arthropod and some vertebrate pests so many fewer can live near your site.

4. Chemical Controls

Chemical control of vectors or pests is not usually a practical option for soldiers, but the use of residual insecticides to treat the interior of semi-permanent or permanent buildings and other selected surfaces can be a very important strategy in some situations. Specific chemicals (*e.g.*, repellents) applied to bed nets, screening, or movable structural elements (like temporary walls or partitions) can greatly reduce or eliminate vector or pest populations in many situations (especially crawling pests and even certain mosquitoes or other biting arthropods). When properly planned and performed, wide area treatments with ULV or fogging devices can knock down a large portion of currently active population of infected vectors, and thus, possibly prevent or greatly reduce local area outbreaks of some diseases (*e.g.*, some relatively deadly encephalitides). Such treatments are beyond the capabilities of most soldiers or tactical units.

5. Preventing tick, chigger and flea bites

When operating in areas infested with ticks, chiggers or fleas, the pants should be bloused into the boots to prevent access to the skin by ticks and other crawling arthropods such as chiggers. Check yourself frequently when walking through tick-infested areas. Upon returning from infested areas, remove all clothing and examine yourself for ticks and chiggers. Infected ticks may require several hours of feeding before pathogens are transmitted. Therefore, personnel who operate in tick-infested areas should check themselves frequently for ticks and remove them as soon as possible. If ticks become attached, the simplest and best method of removal is by a slow, steady pull with a pair of tweezers or forceps. Do not squeeze the body but grasp the tick where the mouthparts enter the skin and pull firmly until the tick is extracted. Be careful not to break off the mouthparts and leave them in the skin. Wipe the bite area with an antiseptic. If hands have touched the tick during removal, wash them thoroughly with soap and water or an antiseptic, since tick secretions may contain pathogens.

Point Source Threats

There are many different potential point source threats that can impact any given deployment. Following are some examples of more common arthropod point source threats and a brief description of their potential impact to military personnel.

1. Spiders

Spiders, in general, are harmless to people. However, a few species are capable of causing serious damage or death in victims.

A. Black Widow Spiders

Black widow spiders, *Latrodectus* spp. are among the most dangerous spiders in the world. They are normally timid, medium-sized spiders (<1 inch long), and shiny black in color. The abdomens are variously marked with red spots or other shapes. The red hour glass on the Southern black widow, *Latrodectus mactans*, is perhaps the most recognized mark among these spiders. Representative of this group of spiders occur worldwide. Other widow spiders of importance occur in

the Middle East, Africa, Asia, and throughout the Western Hemisphere. Other examples include the Brown Widows (cosmotropical, common in the South), Red Widow (Central and southern Florida), and Northern Widow (Northern Florida to southern Canada). These widow spiders should be considered moderately dangerous. Black widows normally will not bite unless provoked or contacted by accident. Toxicity of the venom is highly variable depending on the species.

- Bites are not very painful and may not be felt initially, or there may be slight localized reddening and swelling. However, envenomizations usually result in severe muscular pain, rigid "boardlike" abdominal cramping, tightness of the chest, difficulty breathing, and nausea.

- Black widow bites can be variously misdiagnosed as ruptured ulcer, acute appendicitis, renal ulcer, or food poisoning.

- Mortality rate can be 4-5% without treatment.

- Antivenin is available and useful if used within 3 hours after envenomization

-Treat symptoms that may develop.

B. Brown Recluse Spiders

Brown recluse spiders, *Loxosceles* spp. (recluses or fiddlebacks) are commonly distributed throughout the Americas. The brown recluse, *Loxosceles reclusa*, is perhaps the most recognized member of this group. The fiddle-shaped mark on the cephalothorax, long legs and sleek, brown coloration are characteristic of this group.

- The brown recluse bite is not particularly painful and may not be felt at all. Multiple bites in a single attack are not uncommon.

- The venom is necrotic, and destroys the tissues of the victim. However, not all bites cause necrosis and the extent of necrosis is highly variable among victims ranging from a small "pimple" to severe "craters" that may take months to heal.

C. Tarantulas

Tarantulas are widely feared, but they are not considered to be dangerous. Their bites are similar to a bee or wasp sting. Their fangs are quite large approaching the size of a pair of large needles and bites are quite painful.

2. Scorpions

Scorpions have painful stings and several species can be deadly to humans. Generally, small species with slender claws are the most dangerous, whereas larger species with big claws tend to be less venomous -- however, untrained personnel should never attempt to gauge the danger potential of scorpions based on size alone.

- Most areas of the world have one or more species of particularly dangerous scorpions.

- In Chihuahuan and Sonoran deserts of North America and Mexico, there are only a few dangerous species of scorpion. Stings from these species typically do not swell or redden. Those of most other scorpions in North America do one or both.

3. Honeybee

Honeybee stings kill more people annually around the world than poisonous snakes. Bee stings are painful, but, for most people, that is the only effect. Others however, can have an allergic reaction to the sting and can die from anaphylactic shock. A single bee sting can result in death.

Deaths caused by honeybee stings are due to anaphylactic shock. An initial sting sensitizes the body's immune system, a subsequent sting, which may occur years later, causes shock and sometimes death.

- Africanized honey bees are becoming more widely distributed in the United States since crossing the border from Mexico in the early 1990s. These bees are more aggressive, attack in larger numbers and pursue further, but their venom has no more toxicity than "tamer" races of honey bees.

- If you are “investigated” by a honey bee, do not antagonize it. This could cause the bee to sting and release an “alarm pheromone” which signals other bees to attack.

- If attacked, literally “run for your life”-- get indoors, in a vehicle, tent, any kind of shelter, if possible, and if not, run through brush in a zig-zag pattern to disorient the bees.

- Honey bee stingers are barbed and they, along with the attached venom gland, will remain imbedded in the skin because they are pulled from the bees abdomen following the attack. As long as the stinger remains inserted in the skin, the venom gland will continue to pump venom into the host until the supply is exhausted. To prevent this from occurring, the stinger(s) should be removed as quickly as possible using a straight, sharp edge such as a fingernail, credit card, knife blade or similar tools. Never attempt to remove a stinger with the fingertips as this may actually force more venom into the victim.

4. Fire Ants

Fire ants in the southern United States and southward through South America can be a severe problem because of their unusually large numbers and potent venom. - Their venom is necrotic, like that of the brown recluse spider, but not as potent. Characteristically, a blister forms, the liquid within solidifies and when the blister goes away there is a small pit that may persist several weeks. Normally one does not get just one fire ant sting, and the stings are often quite numerous, because they swarm their victim and release a chemical signaling others to sting, often overwhelming their victim as a result.

5. Wasps and Hornets

Wasps and hornets are present in virtually all areas of the world except the poles. They resemble each other in appearance, and in having painful stings. Unlike honeybees, wasps and hornets have straight stingers and they can sting multiple times. Most stings caused by wasps and hornets only cause pain and are more a nuisance than anything else, and are rarely fatal.

6. Centipedes

Centipedes normally are harmless, but larger species are capable of inflicting painful itching "bites." The "bite is actually produced by the first pair of legs which are modified into claws capable of injecting venom. Centipede "bites" have a characteristic appearance, a series of paired puncture wounds caused by the centipede "chewing" with its poison claws to inject poison.

7. Chiggers

Chiggers are small mites, which insert their mouthparts into pores and inject their saliva. This affects the surrounding cells and causes intense and long-lasting itching. They will characteristically crawl up the body until they reach an area constricted by clothing (sock top, underwear band, etc.) and feed in that area. Throughout Asia, chigger mites are capable of transmitting scrub typhus.

8. Scabies

Scabies mites also cause severe and very long-term itching. The mites burrow beneath the skin surface and live out their life cycles there. Scabies is spread by dermal contact, and they can be found on most parts of the body but most commonly on hands, feet, groin, folds of buttocks and under breasts. The rash caused by scabies is very easy to detect. Scabies may lead to secondary infection if scratched with dirty fingernails (all bites can he infected this way).

9. Biting Bugs

Biting bugs, including wheel bugs, giant water bugs, backswimmers, bed bugs, (*Cimex lectularius*) can inflict very painful bites with their piercing-sucking mouthparts; some can inject non-lethal toxic venom. The pain stops spontaneously in one to four hours. Although such bites are not life-threatening, severe psychological distress may result.

10. Blister Beetles

Blister beetles are not a severe pest in terms of pain and suffering, but they can inflict fairly serious lesions. - Blister beetles are soft-

bodied beetles 1/4 to 1 inch long, in various colors and color patterns, with a well-defined “neck” and “shoulders.” - When crushed against skin, they release a substance that causes a large, painless blister or vesicle. The lesion requires careful management and there is danger of secondary infection. If a blister ruptures additional blistering may occur where the fluid touches the skin. Thus, scratching can lead to extensive damage in some individuals.

11. Urticating insects

Urticating insects, primarily caterpillars that have hollow hairs filled with poison can be encountered around the world. When contacted by humans, hairs embed, break off, and release venom. Venom causes local and sometimes systemic reaction. This produces an urticarial rash. An example is the puss caterpillar. - The lesion from puss caterpillar contact often looks like an outline of the caterpillar. Stings are extremely painful, and the victim may be sick for 2 or 3 days. Some require overnight hospitalization for supportive care. - First aid is to remove hairs with adhesive tape -- stick on the area, then pull off. Then cleanse the wound with alcohol. Secondary exposure of patient care providers does occur and medical staff should wear protective gloves and take care not to come into contact with the hairs.

12. Head and Body Lice

Head and Body Lice have seldom been a problem to troops since World War II, however, the possibility of epidemic typhus is ever-present, especially in refugee situations like those faced during many recent humanitarian assistance deployments. The crab or pubic louse does not vector disease but causes severe itching where it feeds. Proper personnel hygiene and permethrin treated clothing serve to prevent louse infestation among U.S. military forces.

13. Fleas

Fleas can be a problem in areas where dogs and cats roam free in urban areas, and in "wild" areas where wild rodents and their fleas can be contacted. Fleas normally are not a severe nuisance, but can be when they are present in large numbers. Flea bites feel like a strong pin prick, producing reddening, swelling, itching. Site encampments should be located in areas distant from rodent burrows and their associated fleas. **Tunga fleas** (chigoes) found in tropical areas differ from other fleas in that they burrow into the skin, often under toenails. Chigoes should be removed in a sterile manner to prevent secondary infection, which can lead to autoamputation of the digit under extreme conditions like those encountered in contingency conditions.

14. Biting Flies

Biting Flies rank among the most annoying insect pests and can be a severe distraction for military members in an operational environment. For example, **horseflies** and **deerflies** can cause severe biting trauma. They tear a wound into the flesh with their mouthparts and then lap up the blood -- the bite is very painful. In some areas these flies occur in large numbers, thus making outdoor activities difficult. **Stable Flies** (or dog flies) are very persistent, painful biters and they occur throughout most of the Americas. **Black flies** have painful, irritating bites, and because they are fairly strong flyers, they can become a nuisance some distance from breeding sites. **Sand flies**, although small in size, have very irritating bites. **Biting midges** (no-see-ums, sand gnats) arguably are the most irritating of all the small flies that feed on people. They occur periodically, at certain times of the day, but can drive people inside at those times. Some people become very allergic to no-see-um bites and experience reactions that resemble the lesions produced from contact with blister beetle. Mosquitoes can cause serious annoyance problems in addition to spreading diseases, and their bites can produce itchy wheals that can become secondarily infected. **Tsetse flies** can inflict painful bites in addition to being vectors of African sleeping sickness, and they can pose a significant threat to force health during deployments to central Africa.

15. Allergens

Allergens originating from arthropods can cause a multitude of problems. The tussock moth is a hairy caterpillar whose hairs are not venomous but when molting in large numbers, hairs are shed and may be ingested, inhaled, or rubbed into skin, causing severe allergic “hay fever” symptoms.

References and information sources:
Some important published references about vector-borne diseases, their vectors, distributions and prevention include:

Military Publications:
1966. Poisonous snakes of the world, a manual for use by U.S. amphibious forces. NAVMED P-5099, BUMED, Department of the Navy, U.S. Govt. Print. Off., Washington, DC. 212 pp.

1991. Technical Guide (TG) 138. Guide to commensal rodent control. U.S. Army Environmental Hygiene Agency (USAEHA), Aberdeen Proving Ground (APG), MD. 91 pp. This item (in color) can be reached at: www.afpmb.org - select "Publications etc."; then select "Literature Retrieval System" (LRS) = the next to last line on the sub-page; then select "Advanced Search"; then enter the LRS Accession No. "**181762**".

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1995. USACHPPM TG 196. Guide to poisonous and toxic plants. U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM), APG, MD. 77 pp.

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2009. TG 36. Personal protective techniques against insects and other arthropods of military significance. AFPMB. 61 pp. http://www.afpmb.org/coweb/guidance_targets/ppms/TG36/TG36.pdf

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2006. USAF School of Aerospace Medicine. 2006. "United States Air Force Guide to Operational Surveillance of Medically Important Vectors and Pests "Operational Entomology", Version 2.1, 15 Aug. 2006. 116 pp. (available via the AFPMB website at: http://www.afpmb.org/coweb/guidance_targets/vector_and_pestcontrol/Operation_Surveillance_Guide.pdf)

2008. TG 24. Contingency pest management guide. AFPMB, 36 pp. (May).

Non-Military Publications:

Baker, E., T. Evans, D. Gould, W. Hull, and H. Keegan. 1956. A Manual of Parasitic Mites of Medical or Economic Importance. NPCA, Dunn Loring, VA, [reprinted – 1967, by Henry Tripp, Woodhaven, NY]. 170 pp.

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Russell, F. 1983. Snake Venom Poisoning. Scholium Internat'l, Inc., Great Neck, NY. 562 pp.

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Tiroumourougane1, SV., P. Raghava, S. Srinivasan. 2002. Japanese viral encephalitis.
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Pathology

With the frequency of world travel, immigration and refugee populations today, parasitic infections present a growing problem in many areas of the world. A primary concern of health care facilities is the control of parasitic diseases. A successful program for the treatment, control, or eradication of these parasitic infections cannot be realized unless the diagnostic forms of these organisms are rapidly and accurately recovered and identified in the medical laboratory.

a. Proper collection and handling of specimens – essential to ensure parasites will be recovered.

b. Old, inadequate or poorly preserved specimens – of limited value and may lead to inaccurate results.

c. Some medications may interfere with examination

   – Some examples are antacids, antidiarrheal compounds and some antibiotics

d. All specimens should be labeled according to local SOP

   – Minimum information needed for labeling would be **last name**, **first name**, and **full social security number**.

e. Fecal specimens are collected in clean, wide mouthed containers that have a tight-fitting seal.

   1) The container does not have to be sterile

   2) Collect sample directly into container to avoid contamination

   3) Some contaminants such as urine and water may destroy organisms or may contain free living organisms.

Figure 1: Fecal specimen container

NOTE: There are several ways to collect the specimen while avoiding contamination. For example, the patient may initially pass the specimen into a bedpan, plastic trash bag, or newspaper and then transfer a representative portion to the specimen container.

f. Several stool examinations are necessary before parasitic infection can be ruled out.

g. Timely processing of samples is **very important**

1) Specimens can be refrigerated at 3-5 degrees C for no more than 24 hours

2) If specimen cannot be examined within the recommended time frame, it should be preserved in a suitable preservative

NOTE: Stool specimens should **NEVER** be frozen or placed in an incubator

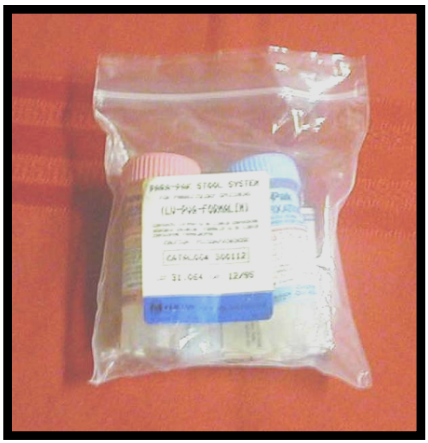


Figure 2: Stool specimen preservation

Stool Specimen Preservation

NOTE: Most laboratories use two vial systems (PVA/Formalin) available from commercial manufacturers

a. Polyvinyl Alcohol (PVA)
    1) Excellent preservative for the morphologic features of intestinal protozoa
b. Formalin preserved specimens
    1) 10% aqueous formalin – best for preparation of most organisms
    2) 5% formalin-saline solution – adequately preserves protozoan cysts, helminth eggs and larvae

Figure 3: Stool preservation kit

NOTE: If the zinc sulfate procedure is to be performed on specimens preserved in formalin, it will be necessary to adjust the specific gravity of the zinc sulfate solution (1.18 to 1.20

c. Merthiolate-Iodine Formalin (MIF) Preparation

1) A combination preservative and stain for fecal specimens

2) Especially useful in field surveys

3) Recovery of intestinal protozoa, helminth eggs and larvae from temporary wet mount preparations

NOTE: Always utilize local SOP or package inserts for instructions on how to use any specimen preservative.

Macroscopic examination of stool specimen

a. Consistency of Stool Specimen – hard, soft or liquid
b. Color – Normal, black, red, gray or chalky
c. Gross blood – Blood present in large amounts
d. Mucus – visible white patches on the stool specimen (should be reported if in excess)
e. Occult blood – Blood present in trace amounts
1) Additional testing need for the detection of occult blood
a) Guaiac test
b) Hemoccult test

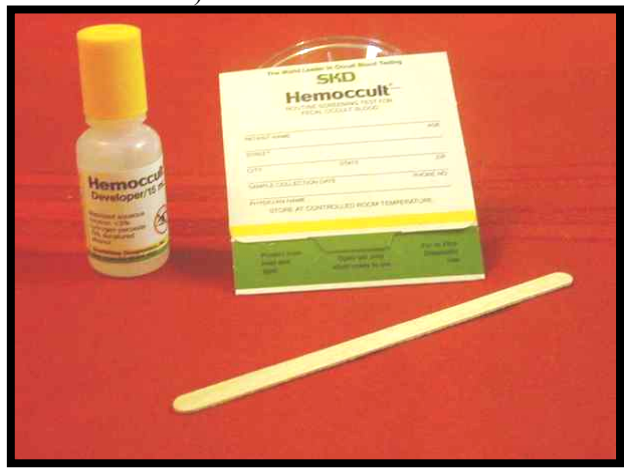


Figure 4: Hemoccult Test

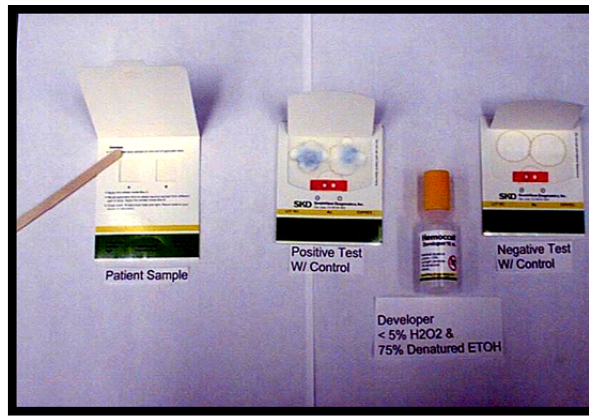
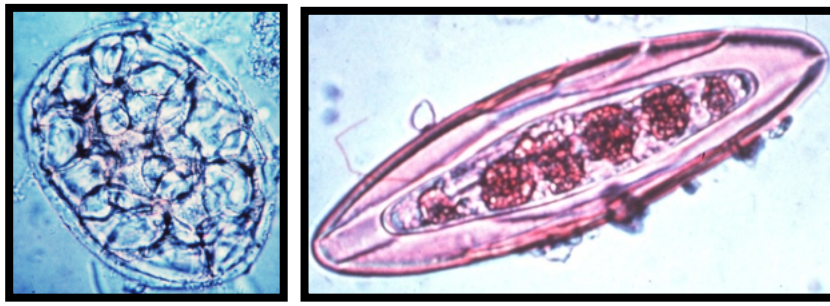


Figure 5: Hemoccult Test

NOTE: The fecal specimen should be observed for the presence of adult helminthes or segments of adult tapeworms, which may occasionally be found on the surface of the sample.

Microscopic Examination

a. Some material may be mistaken for parasites. These objects are often referred to as artifacts.
   1) Examples are animal or plant hair, starch or pollen granules



Figures 6 and 7: Materials that may be mistaken for parasites: Fat Globule (left) and Plant Cell (right)

b. Most widely used procedures for examination of fresh and preserved fecal specimens
   1) Direct wet smears
   2) Flotation or sedimentation concentration procedures
   3) Preparation of permanent-stained smears

c. Direct Wet Mounts
   1) Purpose – recommended for the detection of motile trophozoites from fresh fecal specimens
   2) This method is the simplest method because limited materials are needed

Procedure
- Add on drop of saline to a clean microscope slide
- Obtain specimen on the tip of an applicator stick
- Mix the specimen and saline into a uniform suspension
- Place cover slip over the preparation
- Density – should be thing enough so that fine print can be read through it

NOTE: Intestinal parasites are readily found in direct smears when present in large quantities. However, in most cases, concentration is required for the detection of these parasites. When properly performed, concentration techniques are more reliable by insuring a higher recovery for protozoa and helminthes.

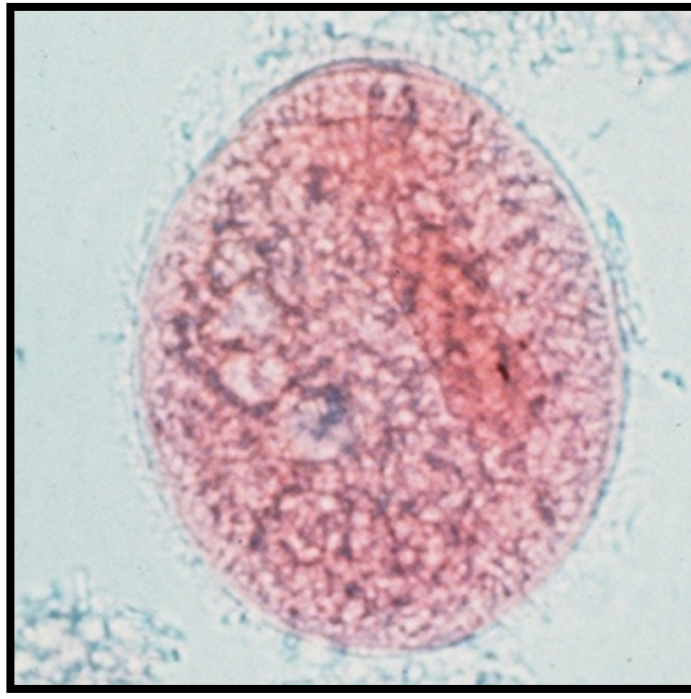


Figure 8: Protozoa species

Capillary Puncture for the Recovery of Blood Parasites

NOTE: Please refer to figure A-1 on page 32 in the Malaria Section

a. A minor surgical procedure, therefore, aseptic technique is a must and all equipment must be sterile
b. Preferred method of collection – when only a small amount of blood is required
c. Simplest method of obtaining blood when making slides for the study of *Plasmodium* spp and other blood parasites

1) Procedure

a) Sites

(1) Finger – preferably ring finger
(2) Ear – lobe of the ear (has higher concentration of white blood cells)
(3) Heel – used on newborn infants

b) Finger Puncture

(1) Warm puncture site to assure good circulation of blood
(2) Cleanse the site to be punctured with alcohol soaked gauze to remove dirt and skin debris
(3) Let alcohol air dry
(4) Hold patient's finger between the thumb and index finger while puncturing and collecting blood
(5) Do not touch puncture site
(6) Puncture the skin by using a quick, firm stroke of the lancet
(7) Collect blood for smears or tests being performed
(8) Apply pressure to the wound using a sterile gauze pad or apply a bandage

c) Precautions

(1) Wipe away the first drop, then place collecting utensils into the blood; do not touch the skin
(2) Avoid squeezing finger near the puncture site (it may shut off the blood supply and introduce tissue fluid)
(3) Have patient look away during finger stick to prevent a reflex action of pulling the arm
(4) Dispose of the blood lancets immediately in a proper receptacle (sharps container)

Appendices

Medical Teleconsultation

Get a second opinion tele-consult from medical specialists by submitting a non-secure email message with relevant attachments (JPEG, ECG, *ect.*):

Teleconsultation Format:

- Used by deployed healthcare professionals
  * Physician, PA, Nurse, SF/DMT, Medic
- No Patient identifying information
- No classified information
- Patient demographics:
  * Age, Branch of Service, Location
- Duration of Problem
- Symptoms Now
  * Getting better? Worse? Staying same?
- Previous treatment & outcome/effectiveness
- Test & results
- Your Diagnosis/Differential Diagnosis
- Limitations to care for patient
  * Medications? Procedures? Evacuation?
- Attachments
  * Digital images, scanned EKG
  * Lab findings, Radiographs
- Include Physician in email
- Consultation answered 7 days/week

Consultations with established contact groups:

Burn Trauma: burntrauma.consult@us.army.mil

Cardiology: cards.consult@us.army.mil

Dermatology: derm.consult@us.army.mil

Infectious Diseases: id.consult@us.army.mil

Nephrology: nephrology.consult@us.army.mil

Ophthalmology: eye.consult@us.army.mil

Pediatrics: picu.consult@us.army.mil

Pediatric Intensive Care: picu.consult@us.army.mil

Preventative Medicine/Occupational Medicine: pmom.consult@us.army.mil

Rheumatology: rheum.consult@us.army.mil

Toxicology: toxicology.consult@us.army.mil

Internal Medicine: im.consult@us.army.mil

Neurology: neuron.consult@us.army.mil

Orthopedics and Podiatry: ortho.consult@us.army.mil

Urology: urology.consult@us.army.mil

Microbiology: microbiology.consult@us.army.mil

Other specialties as requested include: Allergy, Flight Medicine, Endocrinology, EENT, Gastroenterology, General Surgery, Hematology, Legal, Neurosurgery, Nutrition, OB-GYN, Oncology,

Oral Pathology, Pharmacy, Plastic Surgery, Pulmonary Diseases, Psychiatry, Radiology, Speech Pathology, and Vascular Surgery

For consultations for all other specialties email:

Mr. (LTC-Retired) Charles Lappan:

chuck.lappan@us.army.mil

OTSG Telemedicine Teleconsultation

Project Manager

POC:

COL Ron Poropatich, MD

Senior Clinical Advisor

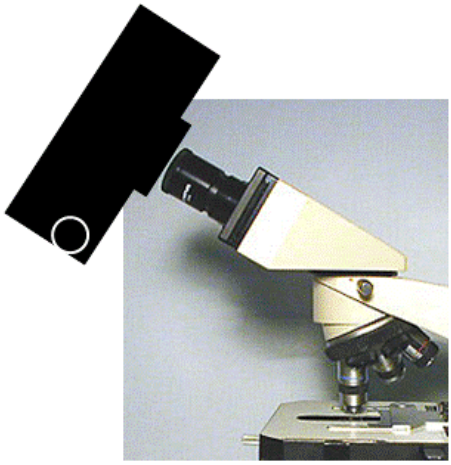
Telemedicine & Advanced Technology Research Center (TATRC)

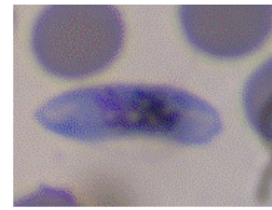
USAMRMC Liason to the Department of Homeland Security

U.S.Army Medical Research and Materiel Command (MRMC)

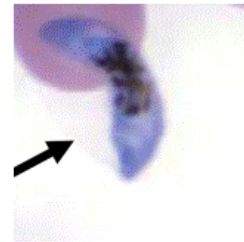
ron.potopatich@amedd.army.mil

Taking pictures through the microscope eyepiece





Picture taken with Canon PowerShot A560 through microscope eyepiece



Picture from CDC Bench aids

Pictures can be taken through microscope eyepieces with ordinary digital cameras that approach the quality of pictures taken with specialized equipment.

1. Focus the specimen by looking through the eyepiece.
2. Using the stage controls, place the specimen in the middle of the field of view. This allows you to rest the camera against the lens. There is a lot of trial and error involved in doing this.
3. Identify several landmarks next to the parasite of interest, such as white blood cells, which will make it easier for you to find the parasite again while looking through the camera.
4. Turn on the camera and turn off the flash.
5. Rest the camera front lens assembly against the eyepiece and find the sample by looking through the camera.
6. Zoom in as appropriate.*
7. Focus the specimen again, looking through the camera while you adjust the microscope.
8. Take several pictures, changing the focus each time.

Whatever you can do to minimize the movement of the camera will improve the sharpness of the picture.

*Many cameras have both digital zoom and optical zoom. Optical zoom uses the camera's lenses to magnify the objects in view, while digital zoom uses electronics to magnify the picture obtained by the lenses. Whether the quality of the picture is degraded or improved by using digital zoom will vary from one type of camera to the next. Try taking pictures with and without using digital zoom.

Useful Websites

Course website:
http://wrair-www.army.mil/OtherServices_TropicalMedicine.aspx

The course website includes ALL of the material presented in this manual as well as other course materials and helpful information.

WRAIR Homepage
http://wrair-www.army.mil/

Some websites that contain details about vector-borne human diseases, their vectors, distributions and prevention include:

The Acarological Society of Amer. (ASA) web site info/ACARI (mites & Ticks)
www.Acariweb.com/ASA/

AFPMB website: www.afpmb.org (some suggested specific references)
http://www.afpmb.org/pubs/Field_Guide/field_guide.htm
http://www.afpmb.org/pubs/Living_Hazards/living_hazards.htm
http://www.afpmb.org/dveps/dveps.htm (then select the ones of interest)
http://www.afpmb.org/coweb/guidance_targets/ppms/TG36/TG36.pdf

American Arachnological Society at: www.americanarachnology.org

The CIA Factbook: http://www.cia.gov/library/publications/the-world-factbook/geos/uv.html

U.S. Centers for Disease Control and Prevention (CDC): www.cdc.gov (search by topic)

The U.S. CDC, Travelers' Health, "searchable" on-line reference (Yellow Book, 2008) is at:
http://wwwn.cdc.gov/travel/contentYellowBookAbout.aspx

Link to NCMI website (MEDIC, copy/info):
https://www.intelink.gov/ncmi/medic_downloadable.php

Link for the New Reptile Database: http://www.reptiliaweb.org

Pan Amer. J. Publ. Hlth in e-format 4 free & open access at: http://journal.paho.org & http://www.scielo.org

The Scorpion Files at: http://www.ub.ntnu.no/scorpion-files/

Toxinology website, Adelaide, Australia at: www.toxinology.com

Walter Reed Biosystematics Unit at: www.wrbu.org

The World Health Organization (WHO): www.who.int

WHO Pesticide Evaluation Scheme (WHOPES): http://www.who.int/whopes

WHO searchable Snake/Antivenin Database (still under development): http://apps.who.int/bloodproducts/snakeantivenoms/database/

Other websites:
Mycology photos: www.doctorfungus.org

CDC Parasite pages: http://www.cdc.gov/parasites/

Shipping of Microbiology Specimens
http://www.cdc.gov/nczved/divisions/dvbid/specimen/bacterial_shipping.html#shipping
http://www.mamc.amedd.army.mil/pathology/TestIndex.htm

Biosafetly in Microbiological and Biomedical Laboratories (BMBL) 5th Edition http://www.cdc.gov/biosafety/publications/bmbl5/index.htm

MEDLINET (AMEDD Virtual Library): https://medlinet.amedd.army.mil/

USUHS Homepage: http://www.usuhs.mil/

GEIS Homepage: http://www.geis.fhp.osd.mil/

National Center for Medical Intelligence (NCMI)
https://www.intelink.gov/ncmi/index.php

DoD Clearance Guide for travel
https://www.fcg.pentagon.mil/

Useful Websites for Malaria:

Malaria diagnostics
CDC DPDx http://www.dpd.cdc.gov/dpdx/html/malaria.htm

WHO
http://www.who.int/malaria/publications/microscopy/en/index.html
The following books can be downloaded in PDF form from the WHO Website:
Basic Malaria Microscopy. Part I. Learner's Guide. 2nd ed. World Health Organization, Geneva. 2010
Basic Malaria Microscopy. Part II. Tutor's Guide. 2nd ed. World Health Organization, Geneva. 2010

Royal Perth Hospital: http://www.rph.wa.gov.au/malaria.html
This site includes a 90-picture interactive training program for *Plasmodium* species identification.

Johns Hopkins Malaria Research Institute: http://malaria.jhsph.edu/

Evaluation of Malaria Rapid Diagnostic Tests
WHO http://www.wpro.who.int/sites/rdt

Microscopy
Olympus http://www.olympusmicro.com/

Abramowitz, M.: *Microscope. Basics and Beyond*. Olympus America Inc., Melville, NY.
2003. This book can be downloaded free as a 20-MB PDF file from the following Website:
http://micro.magnet.fsu.edu/primer/index.html

Diagnosis of Malaria
Bench Aids for Malaria Microscopy. 3rd ed. World Health Organization, Geneva. 2010. Available in the U.S. from Stylus Publishing, Herndon, VA. A scanned version of the second edition is available free online at:
http://www.who.int/malaria/publications/atoz/9241545240/en/

Frequently Used NSN's

ITEM	NSN	UI	REMARKS
Consolidated Field Sanitation Kit	4540-01-578-4352	set	
Bednet, Pop-pop up, Coyote Brown *USE CODE 26 (Combo of 2B and 2L)	3740-01-518-7310	ea	Must order through CL IX (SARSS)
Insect Repellent w/sunscreen (DEET w/sunscreen)	6840-01-288-2188	12 tubes/bx	
Insect Repellent, personal application (DEET)	6840-01-284-3982	12 tubes/bx	
Insect Repellent, Clothing Application (IDA Kit)	6840-01-345-0237	12 kits/bx	
Insect Repellent, Aerosol (for uniforms or bed nets)	6840-01-278-1336	12 cans/bx	
Insect Repellent, Clothing Application (Concentrated Liquid)	6840-01-334-2666	12 btl/bx	
Insecticide, d-Phenothrin, Aerosol	6840-01-412-4634	12 cans/bx	
Demand Pestab	6840-01-431-3357	1 unit 40 tabs	
Sprayer, Pesticide, 2 gal SS tank	3740-00-641-4719	ea	
Wasp Freeze	6840-00-459-2443	12 cans/bx	
Max Force Granular Fly Bait`	6840-01-518-5807	tub (5 lb)	
Revenge Fly Catchers (Hanging sticky tape)	3740-01-240-6170	case	
Flies Be Gone Fly Bags	3740-01-523-0708	cs/50	
Victor Fly Strips (sticky traps)	3740-01-412-9371	cs/144	
Insect trap , Gold Stick fly trap	3740-01-542-9591	bx/24	
Mouse snap traps	3740-00-252-3384	pkg/6	
Container, Rodent Bait Plastic (Rat)	3740-01-481-1313	bx/6	
Container, Rodent Bait Plastic (Mouse)	3740-01-481-1312		
Rodent Bait (Talon G pellets in small bags)	6840-01-508-6085	bucket	
Rat snap traps	3740-00-260-1398		
Trap, Rodent Glue	3740-01-240-6170	bx/24	
Vec-Test Malaria Rapid Assay (looking for malaria in the mosquito)	6650-01-551-5327	kt	
Wall Mount, 80 Watt Fly Light (food service friendly)	3740-01-286-2361	ea	
Wall Mount, 40 Watt Fly Light	3740-01-286-2362	ea	
Fly Swatter	3740-00-252-3383	pk/12	

Plug, Ear, Hearing Protection, Universal Size, Disposable, 400s	6515-00-137-6345	pg	
Purell Instant Hand Sanitizer, 3 oz bottle, olive drab	6508-01-5355409	24 btl/case	
Wag Bag Waste Kit	4510-01-485-0760	case of 100	http://www.goclean waste.com/wag-bag
Wag Bag Waste Kit	4510-01-485-0759	case of 12	http://www.goclean waste.com/wag-bag
Go Anywhere Portable Toilet	4510-01-485-0736	1 ea	http://www.goclean waste.com/products #pop
Shower Pail, Collapsible	8465-00-935-6649	ea	
5 gal Water Can	7240-00-089-3827	ea	
Mioxx Pens (individual water purification)	4610-01-513-8498	ea	
Rubbermaid Orange Drinking Water Cooler	7330-01-449-2319	ea	
Hand Washing Station	7360-01-480-8487	ea	
Chlorination Kit, Water (100 ampules, DPD 1 tabs etc)	6850-00-270-6225	kit	
Calcium Hypochlorite, 5 lb bottle	6840-00-238-8115	btl	
Calcium Hypochlorite, 6x16 oz bottles	6840-01-358-4336	bx	
Calcium Hypochlorite, 12 x 3.75 lb bottles	6840-00-242-4770	bx	
Calcium Hypochlorite, 16 oz bottle	6810-00-255-0471	btl	
Chlorine Test Strips	6550-08-133-2361	btl/50	
Water Test Strips (HACH 9 in 1)	6550-08-133-2443	btl/50	
Water Test Strips (HACH 5 in 1)			
Water Test Kit M272	6665-01-134-0885	ea	
Paddle Tester, Total Aerobic Bacteria/Disinfection Control		pk/10	www.hach.com
Paddle Tester, Total Aerobic Bacteria/Yeast and Mold		pk/10	www.hach.com
Light ultraviolet, specimen examining (hand held black light)	6530-01-451-5144	ea	
Water Purification Tablets, Iodine	6850-00-985-7166	cs/100 btl	
Respirator, Air Filtering, Infection Control (N95)	6532-01-439-8571	case of 120	
Mask Respirator N95	6515-01-533-8138	pkg of 20	
Binax Now Malaria Rapid Assay	6550-01-554-8536	12-tests/box	http://www.binaxnow.com/malaria.aspx
Binax Now Malaria Rapid Assay	6550-01-554-8731	25-tests/box	http://www.binaxnow.com/malaria.aspx

Stay Alert Gum	8925-01-530-1219	Bx/24